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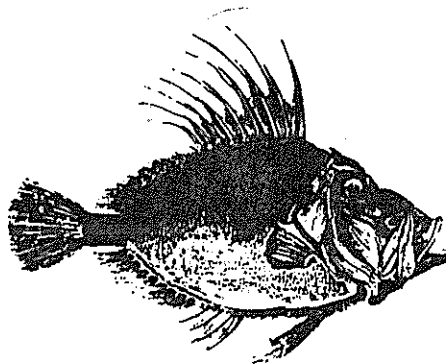
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of the  
FOURTH ANNUAL TROPICAL AND SUBTROPICAL  
FISHERIES TECHNOLOGICAL CONFERENCE OF THE AMERICAS  
April 22-25, 1979  
St. Petersburg, Florida



Compiled by  
Ranzell Nickelson II

The Tropical and Subtropical Fisheries Technological Society of the Americas is a professional and educational association of fishery technologists interested in the application of science to the unique problems of production, processing, packaging, distribution and utilization of tropical and subtropical fishery species.

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THE INTERNATIONAL FISHERIES SCENE - CHANGING  
DIRECTIONS AND NEW PRIORITIES

by

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Changes Resulting from the New Regime of the Sea

I wish to start by painting a rough picture of the international fisheries position, with a broad brush, and then to attempt to define the impact of changes that are taking place on the demand for technology.

Preliminary indications are that the world catch for 1978 increased by 1.5 percent over that of 1977 to a level of 75 million t, thus equalling the record catch of 1976. The factor responsible for the lower total catch in 1977 was the changes in jurisdiction resulting from general adoption of 200-mi Extended Economic Zones (EEZ's). While this perturbation of total catch levels seems to have been resolved, the composition of the catch and the countries participating in it have changed markedly. While some of these changes are due to reduced or increased biological availability of the resource there have also been political implications. These two factors have perhaps been more strongly felt than alterations in demand, although effects on patterns of trade have been substantial.

White fish catches have increased, due principally to higher landings of hakes from the South Atlantic, Chile and Peru (these species are called hake everywhere else except in the U.S.A. where they are known as whiting). The South African pilchard catch was reduced from 300 000 to about 40 000 t but elsewhere sardine landings increased and more use was made of this species for human consumption. For instance, South Africa imported canned and frozen sardines from Peru to make up for lost production. Mackerel catches were also increased with the species mainly in demand in Africa and Eastern Europe. There is, however, a tendency for mackerel with high fat content to become more accepted in Europe and the U.S.A., as a herring substitute.

Shrimp landings were good and shrimp continued to be in high demand. Salmon catches in Alaska were good but in Japan were much reduced. Japanese importers were therefore in the market for frozen Alaskan fish but elsewhere demand was slack because of the botulism incident in the U.K. resulting from imported U.S. product.

The effect of changes in the regime of the seas caused a further reduction in the share taken by the centrally planned economies, especially the U.S.S.R. Spanish catches also decreased again for the same reason while those of the U.S.A. rose and more attention was paid to exports by that country.

International trade increased sharply, mainly ascribed to changes in EEZ's, there being an additional 300 000 t of fish moving in the international market place during the last year. The 14 percent increase in exports brings them to a level of U.S.\$ 6 735 million on a worldwide basis. The trade between countries has inevitably been affected by currency fluctuations, particularly the declining value of the U.S. dollar and the appreciating yen. The U.S.A. and Japan are still the major seafood importers with the Japanese showing a faster rate of growth as a result of the rising yen. Exports came from a greater number of sources, particularly developing countries, which in itself creates additional difficulties for regulating agencies in importing countries.

The negotiation of fisheries agreements and the formation of joint venture companies have been a major feature of the recent past. All sorts of joint venture companies have been set up, such as government to government, government with private partners and vice versa. Degree of foreign ownership and control are also very different from place to place. It is normal for the partner with the resource, generally a developing country, to seek the technology, finance and catching capacity from one of the developed countries with a substantial fishing industry. An example of this is the linkage between Argentina and Fed. Rep. of Germany. However, there are many anomalies, such as the joint venture between Sri Lanka and Nigeria to catch mackerel in Sri Lankan waters for the Nigerian market. At the same time Nigeria is making joint venture agreements with other foreign partners to exploit her own waters and a substantial unsatisfied demand for fish exists in Sri Lanka. Situations, such as this, emphasize that there is no fixed pattern. For the technologist this implies that there can be no standard response. However, the need for technology has definitely been sharpened by recent events and I expect this demand to be sustained. As a result there is a vital need for more trained food technologists to be involved in the fishing industries, both of developed and developing countries.

#### Technological Inputs to Meet New Challenges

Rejection of fishery products by the regulatory agencies of importing countries is a very severe problem. These rejections result in substantial financial losses, particularly in developing countries, and countries with a history of high rejections often find that prices they are offered are lower than ruling world market values. Although attempts are being made to improve quality control and export certification practices there is no evidence of any sustained decrease in rejections. With the pattern of trade changed toward a greater number of suppliers from more countries it is likely that problems will be encountered by enforcement agents in exporting and importing countries. Methods need to be improved and rationalized, but above all there is a requirement to feed back to the producers information on overcoming their problems. Experience has shown that the main problem is contamination and/or spoilage of raw material before it enters processing plants. All aspects of quality assurance, from the commercial as well as the public health points of view merit more attention from quality controllers and fish inspectors. In the world at large there are not enough trained people to fulfill these functions, a need particularly apparent in developing countries.

A further effect on EEZ changes is shown in resource utilization. In the U.S.A. and Canada this is bringing about radical new developments in the fishing industry. In many developing countries, where the level of technical expertise

is much lower, it is creating grave problems. The need for rational management practices is implied when countries lay claim to extended zones of economic influence. The new regime of the seas provides magnificent opportunities for the introduction of reliable resource appraisal techniques and responsible approaches to resource management. We will have to hope that governments do not neglect these opportunities.

Apart from the species in demand in the international market place there are new opportunities to commercialize those species which are at present under-utilized. These may have been neglected previously as it was cheaper for the countries now controlling the resources to catch species of greater appeal to them in somebody else's waters. An example is the declining British cod catch in distant waters and its possible replacement with the blue whiting.

Conversely there are many countries that find that they have laid claim to species not known, or not in demand, in their own countries but which they would like to use for the nutritional benefit of their own people, as well as for export. Examples are hake resources in Peru and small pelagic species in Mexico. Making use of these resources requires the introduction of new, and often complex, industrial infrastructures for catching, handling, processing and marketing, with all the attendant technological needs.

One problem which continues unabated by EEZ changes is the extremely severe loss of useful protein caused by discard of shrimp trawler by-catches. With demand for fish projected to rise steeply it is essential to continue to look for technological solutions to the problem which could also be economically feasible. As regards immediate increases in availability of fish, these can be brought about by post-harvest loss prevention. Estimates of losses by spoilage, infestation by insects, etc. are difficult to make but dried fish losses in developing countries are, for instance, conservatively estimated as 30 percent. Insect attack is the major cause.

There is clear evidence that latent quantities of conventional species, are unlikely to be found. The implication is that in order to satisfy demand it will be necessary to utilize unconventional species, which means that all the technology required for this purpose must be developed, as it is presently nonexistent. Without discussing the availability of these resources, suffice it to say that they are sufficient to merit development of technology. What people who talk glibly of "marine resources" often fail to recognize are the technological inputs required to convert them into palatable human food.

The foregoing is an indication of some major areas which demand increased attention from technologists, both now and in the foreseeable future. The international agencies are working to upgrade technological levels and a few selected examples of FAO activities are given below.

#### FAO Responses to Changing Conditions

The Fisheries Department of FAO has reacted to the recent changes in a number of ways, although much of the programme was already directed to helping developing countries become self-sufficient in the exploitation and use of their fish resources.

A strong programme to offer advice to Member Governments on new strategies and policies which they can adopt in their new EEZ's has been set up. This will

initially be funded by a trust fund from the Norwegian Government and will offer assistance with new policies such as management measures, quota assessments, licence limitation, etc. At the same time the commercial side will be covered by assistance with the planning of joint ventures and in some cases introduction of partners. On the technological and marketing side assistance with quality assurance and identification of markets for products from developing countries will be available.

A market intelligence service for Latin American countries (INFOPESCA) has been set up with headquarters in Panama. The objective is to provide channels of communication between producers and potential customers, and to stimulate a more diversified and higher quality product range. The project is enjoying remarkable success and during its first year of operation was responsible for the initiation of new trade in fish products of about 90 000 t valued at U.S.\$ 60 million. In the future it is hoped to graft on a quality assurance segment to this project to advise producers on measures necessary to meet the quality guidelines of regulatory agencies and buyers.

Similar market intelligence services are at different levels of preparation in Asia, Africa and the Middle East. The ultimate goal is to create a global network covering both developed and developing countries.

A programme for making use of shrimp trawler by-catch is being prepared and will be carried out as a joint activity with SELA (Sistema Económico Latinoamericano). Coordination with activities elsewhere will be assured and it is hoped that a number of alternative solutions will be investigated and tested. Considerable attention is being paid through a number of field projects and collaborative programmes to post-harvest loss reduction. Improved solar driers are being developed and tested, and guidelines for minimum equipment needs to ensure proper fish handling are being drawn up.

Two major global projects are underway to improve the handling and utilization of small pelagic species, funded by the Danish and Norwegian Governments respectively. These projects are seen as preparation for an attack on all aspects of utilization of nonconventional resources in the future, for which support is being sought. It is estimated that about 40 million t of small pelagic species could be used for human consumption, but only if the raw material can be landed in sufficiently good quality, can products be developed and markets established. The opportunity to investigate the prospects is challenging but the magnitude of the problem makes it a little daunting. The nutritional rewards, however, are potentially great as the products are urgently required in food deficient countries.

A good proportion of the small pelagic species currently being caught are destined for fish meal and oil production. If efforts to conserve them for human consumption are successful raw material shortages will be felt by the fish meal industry. The most obvious resources available as a replacement, are the meso-pelagic species, the lantern fish, which are estimated to be present in tens of millions of tons. We are working to develop methods of capture and processing techniques for these species.

These projects and many others are operated by FAO either by direct assistance to a member country or within a regional framework. The Fish Utilization and Marketing Service of FAO has developed a network of collaboration between technological institutes in developing as well as developed countries. This was started in

Asia and has spread to Africa and Latin America. The principle is to combine resources to fight common problems with the objective of making the developing partners self-sufficient in the adaptation and application of technology. Networks such as this have to grow slowly if they are to remain strong. We are always on the look out for new partners and collaborators and very anxious to enter into correspondence with interested individuals or organizations.

PRODUCT QUALITY AND SAFETY RESEARCH ACTIVITIES  
OF THE NATIONAL MARINE FISHERIES SERVICE

Thomas J. Billy and E. Spencer Garrett  
Seafood Quality and Inspection Division  
National Marine Fisheries Service (NMFS)

It is indeed a pleasure to be here today and describe briefly some of our activities this year in the fishery product quality and safety research areas. I think it is generally not recognized that we have a sizable program in this particular area of research. During fiscal year 1979, for example, we are expending \$540,000 and more than 123 man-years of effort.

Obviously, time does not permit a detailed review of all the ongoing projects, so what I intend to do is take you on a quick tour around the country and discuss the principal projects by facility location. Should you want further detailed information on any of these projects, I invite you to contact the laboratory directly (a list is provided at the end of the paper).

UTILIZATION RESEARCH DIVISION  
SEATTLE, WASHINGTON

Beginning in the Pacific Northwest, our Seattle facility is engaged in numerous product quality and safety activities. Regarding product quality, studies are underway comparing the physical and chemical changes of fish held in ice with other holding systems such as modified refrigerated seawater (MRSW) systems. Principal species involved are Pacific cod and pollock.

Onboard freezing studies of raw sections of King Crab are underway to extend fishing time. A process employing citric acid treatment with a short blanch (1 min at 212°F) and rapid freezing seems to have merit. These particular studies are being done at our Kodiak, Alaska, facility, which is part of the Utilization Research Division.

Additionally, chemical studies are ongoing at Seattle to further correlate trimethylamine (TMA), dimethylamine (DMA), and formaldehyde as quality assessment tools. Principal species being emphasized are pollock, Pacific cod, halibut, sole, and rockfish. They are also doing some DMA inhibition studies on several gadoid species using antioxidants such as tertiarybutylhydroquinone (TBHQ). Ethylenediaminetetraacetic acid (EDTA) strongly accelerates DMA formation in gadoid species.

The product safety research conducted at the Seattle Laboratory includes completion of the dieldrin/endrin survey of red snapper, king mackerel, and Spanish mackerel in the Gulf and South Atlantic. At this report, none of these species appear to represent a potential problem. Species from the Pacific Northwest will be studied next.

In terms of inorganics, special focus is being devoted to the significance of lead, arsenic, and cadmium in sediments, water, and food chain organisms, and how they are affected by season, area, handling, and processing procedures for food fish such as halibut.

On the microbiological side, both toxigenicity and applied studies relating to Clostridium botulinum are continuing. Among other things, the toxigenicity studies are aimed at defining the role of natural inhibitors. The applied work deals with more precisely defining the sodium nitrite and sodium chloride requirements for botulinum inhibition in hot smoked salmon, sablefish, and whitefish, and to develop alternate processing parameters for Good Manufacturing Practices (GMPs).

GLOUCESTER LABORATORY  
GLOUCESTER, MASSACHUSETTS

Moving across the country to New England we find our Gloucester facility, which is engaged in both product quality and safety research activities.

Storage studies are underway for relating quality assessment to biochemical and organoleptic changes on a number of products, including blue mussel (Mytilus edulis), roller extracted pasteurized blue crab meat, re-formed lump meat, Antarctic krill, South American whiting (block form), red hake fillet blocks, and stringray.

Our product quality grade standards program is located in Gloucester. In terms of standards, three are soon to be published--fresh and frozen fillets, frozen minced fish blocks, and frozen raw breaded and fried scallops. The unified shrimp standard is soon to be put on display. This program is also now responsible for developing or making changes to military purchasing specifications used for Department of Defense purchases.

Relative to product safety, the major emphasis has been focused on measuring the nitrosamines, if any, present in smoked sablefish, kippered salmon, nova lox, lox, chub, butterfish, mackerel, and sturgeon samples prepared by the Seattle laboratory.

SEAFOOD QUALITY AND INSPECTION DIVISION OFFICE  
WASHINGTON, D.C.

The headquarters unit in Washington, D.C. coordinates all of NMFS's product quality, safety, standards, and compliance activities; directs the Department of Commerce fishery products inspection program; provides liaison with other federal agencies, trade

associations, etc.; and handles most of our international activities. Some of its activities are now summarized.

First is the nomenclature project which is trying to bring some systematic order to naming and labeling fishery products. The important point to remember is that it is product (i.e., market) names with which we are dealing and not new common names for fish species. It is encouraging to note that FDA Commissioner Kennedy has recently agreed with the need, concept, and general approach in this matter. Research is now underway under contract to develop objective test methods for measuring the eight key edibility characteristics which will form the basis for the new system.

Another project relates to determining the cause and significance of histamine formation in tuna during handling aboard vessel or processing into canned products.

Additionally, our headquarters unit has been responsible for the "Apparent Risk Modeling" activities for the microconstituents program. This involves taking the contaminant data base and overlaying U.S. consumption patterns to determine intake by populations at risk. This has been done for mercury, and in large part served as a basis for FDA increasing the mercury action level from .5 ppm to the current 1.0 ppm. The computer simulation model is now being applied to cadmium residues. This activity recently has been transferred to our Charleston facility, although some of our headquarters staff are still directly involved in the project.

#### CHARLESTON LABORATORY CHARLESTON, SOUTH CAROLINA

This laboratory, which was relocated from College Park, Maryland, has many programs and projects in product quality and safety research.

This is where the national microconstituents program is located. During fiscal year 1979, several objectives are being addressed, including improving analytical methods for determining trace elements in fishery products, determining polychlorinated biphenyls (PCBs) and related compounds in selected fishery products, accelerating research on the mode of occurrence and chemical form of cadmium in shellfish, and modifying the current computerized contaminant data bank system to accept other classes of data such as fishery product nutrient composition. This data bank presently contains information on 15 trace metals in over 200 commercial species of fish.

Additionally, there are some ongoing collaborative studies underway on methylmercury methodology. Collaborative studies are just going on stream with the University of California, Berkeley, to determine the effects of selenium on methylmercury disposition in various animals. There is a cooperative effort underway with the National Fish Meal and Oil Association to provide FDA with the information necessary to upgrade hydrogenated marine oils for



human consumption in the U.S. Finally, an inoculated pack study is underway relative to the time/temperature parameters necessary to destroy botulinum in pasteurized oysters. This pasteurization process was developed by the Charleston staff to increase the utilization and shelf life of oyster products.

With regard to product quality, there are several projects.

Information is being developed on composition and potential shelf life of several underutilized southeast species, with special emphasis on high lipid fish such as thread herring and Spanish sardines.

There is an ongoing minced fish project, which in times past has been limited to the single species concept using croaker, trout, or other sciaenids. This is being refocused on the mixed species concept (i.e., mixing more than one species in the frozen minced fish block).

Another project is devoted to preliminary screening of underutilized species for export market potential, emphasizing smoked, salted, fermented, and other desired processed forms.

As part of the export study, a small project is being initiated to address the quality problems associated with handling large fin-fish catches in the southeast by using refrigerated seawater (RSW), chilled seawater, etc.

#### NATIONAL SEAFOOD QUALITY AND INSPECTION LABORATORY PASCAGOULA, MISSISSIPPI

Finally, we go to Pascagoula where we find our National Seafood Quality and Inspection Laboratory. This facility provides the principal laboratory support for our inspection program for fishery products. This mission involves research, monitoring, and training activities.

With regard to research, the largest survey ever conducted of canned tuna products for extraneous material has just been completed. This will provide a baseline upon which to base tolerances for such defects. As this is winding down, a Toxic Organic Substances Survey (TOSS) of imported fishery products subjected to USDC inspection is being implemented. The reason for the survey becomes clear if you look at the principal countries to which we export pesticides that are banned in the U.S. You will find that they are among the leading countries from which we import fishery products.

Development of "Dip Stick Methods" which are rapid and can serve as a useful screen by our in-plant inspectors is another project area. This year the laboratory is concentrating on methods for histamine and Salmonella determinations.

There is also a project in cooperation with the National Fish Meal and Oil Association to determine the chlorine break points and alternatives to the chlorination of fish holds.

On the monitoring side of the laboratory, products which represent a high potential risk are under microbiological surveillance. All new products are subjected to bacteriological and chemical analysis as well.

This is generally the first facility that engages in "hot sample analysis"; that is, analyzing products that have been reported to be implicated in a foodborne outbreak of disease.

Finally, this facility has the training responsibility for the USDC inspection program and also engages in training state food inspectors. In this regard, they have just completed a training course for the State of Georgia food inspectors and will soon do so for the State of Louisiana.

#### ADDITIONAL ACTIVITIES

Aside from these research activities, our agency engages in several other important activities dealing with product quality and safety.

First of all, we enforce the Lacey Act which gives NMFS authority for the search, seizure, and arrest of persons who have violated state laws and have subsequently moved the violative product into interstate commerce. This generally involves quite a bit of undercover activity on the part of our enforcement agents. This little known authority recently was used to gain criminal and civil prosecutions in Florida and South Carolina of persons who were illegally transporting contaminated molluscan shellfish from polluted waters.

Also, we have an operational field force of some 80 inspectors scattered throughout the United States, Puerto Rico, and American Samoa. These inspectors, in cooperation with participating processors, certified over 600 million pounds of seafood as to wholesomeness and quality in 1978.

#### CONCLUSION

In conclusion, the bottom line of all these varied activities is that NMFS is assisting the U.S. seafood industry in meaningful fashion to produce safe and nutritious products that consumers can purchase with confidence.

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### INTRODUCTION

From July through November of 1978, members of The University of Georgia Marine Extension Service Seafood Technology Program conducted a study designed to assess the effectiveness of three different inexpensive and easily performed clean-up or sanitization methods as applied to several product handling and storage surfaces aboard a commercial shrimp boat. The recommended clean-up or sanitization methods tested during the performance of this study were derived from several sources: (i) Carroll et al. (2), (ii) Codex Alimentarius Commission (3), (iii) Lane (8), (iv) Nickelson (9), (v) State of Alaska (10), and (vi) Williams (11).

In addition to enumerating surface microbial loads following routine product handling and the reductions in microbial numbers effected by the experimental clean-up or sanitization procedures, another portion of the study related surface microbial quality to the storage stability of shrimp held on ice (0° C) for 15 days.

A commercial shrimping vessel, the "Miss Kathy" (60' wooden hull, sealed and fiberglassed rear deck), was chosen as being representative of the majority of the 398 Georgia vessels licensed for commercial shrimping during the 1978 season, and was used exclusively throughout the course of this investigation (see Appendix Figure 1 and Appendix Table 1).

### MATERIALS AND METHODS

The three different clean-up or sanitization methods tested were:

- 1.) Sea water rinse (traditional);

- 2.) Sea water rinse followed by scrubbing with powdered laundry detergent, followed by another sea water rinse; and,
- 3.) Sea water rinse followed by scrubbing with powdered laundry detergent, followed by a second sea water rinse, followed by scrubbing with chlorine bleach (5.25% sodium hypochlorite solution diluted to 1600 ppm when mixed with sea water on the surfaces), and a final rinse (8).

The three shrimp boat surfaces examined for existing microbial contamination and reductions of surface contamination effected by the three aforementioned methods were: (i) a sealed wooden deck covered with fiberglass, (ii) an unfinished wooden sorting/heading table, and (iii) plastic storage coolers.

Deck and sorting/heading table surfaces were sampled at three different intervals during the daily shrimping operation: (i) before fishing, (ii) after fishing, and (iii) after clean-up or disinfection. Storage cooler surfaces were sampled only twice during the daily shrimping operation (before fishing and after fishing).

Each experimental surface area of 25 square centimeters was sampled by the wet/dry swab method (Baldock, 1974)(1), (Favero et al., 1968)(4). The sample swabs were then placed in sterile phosphate buffer solution and held and transported on ice (0° C) until returned to The Marine Extension Service Laboratory in Brunswick, Georgia for analyses (5).

Three swab trials were performed for each of the aforementioned sampling conditions. All swab trial microbiological analyses were performed in duplicate:

- 1.) Total (aerobic) Plate Counts (TPC)(5);
- 2.) Total Coliforms (MPN Three-Tube Technique)(5);  
and,
- 3.) Fecal Coliforms (MPN Three-Tube Technique)(5).

In order to provide information about the microbial condition of Georgia shrimp prior to any human contact, samples of shrimp were taken directly from the net in an aseptic manner (sterilized tongs), placed in sterile Whirl-Pak bags, and stored on ice. These shrimp were not headed. Samples of shrimp handled on or contacting the three aforementioned surface areas cleaned or sanitized by experimental methods 1, 2, and 3 were mixed thoroughly with and held on ice. These "handled" shrimp were headed prior to iced storage.

Upon return to The Marine Extension Service Laboratory, all "handled" shrimp samples were removed from their temporary storage containers and segregated into two lots. One lot was used for immediate (Day 1) microbiological analyses and the other for succeeding storage stability experiments. Sub-samples of shrimp from the second lot were removed after 3, 6, 9, 12, and 15 days of experimental storage (0° C, on ice, at least 2 lb ice per lb of shrimp, with adequate drainage) for microbiological analyses. All shrimp microbiological analyses were performed in duplicate (TPC, MPN Total Coliforms, and MPN Fecal Coliforms).

## RESULTS AND DISCUSSION

The present study revealed that, after fishing, sorting, and handling, the fibreglassed rear deck of the commercial shrimp boat sampled had an average TPC of 35,000 organisms/cm<sup>2</sup> present before the commencement of any clean-up or disinfection procedures, either "traditional" or experimental. However, rinsing with sea water effectively removed 91.2% of the organisms originally present. Scrubbing the deck with powdered laundry detergent reduced the deck surface microbial load by an additional 4.8%, and the application of bleach further reduced the deck TPC by 3.9%. Thus, a 99.9% removal of the microbial population originally present was achieved by the combination of clean-up and disinfection steps 1, 2, and 3 (see Figure 1).

Following sorting and heading, the unfinished wooden sorting/heading table was found to have an average TPC of 93,000 organisms/cm<sup>2</sup> originally present. Although sea water was somewhat less effective on the table, it nonetheless removed 80.6% of the original microbial load. However, scrubbing with laundry detergent caused a temporary seven-fold increase in table TPC's. The slightly reduced effectiveness of the sea water rinse and the increase in microbial numbers observed after the detergent scrub apparently were related to the rough, porous nature of the unfinished wood, which allowed for the accumulation of microbes. The emulsifying action of the detergent caused the microbial contaminants to be floated out of the wood grain, making them available for treatment with sanitizer. Upon application of bleach, 99.9% of the microorganisms originally present were destroyed (see Figure 2).

After iced shrimp were removed from the plastic storage coolers used by the commercial shrimp boat, the cooler surfaces were found to have an average TPC of 1,340 organisms/cm<sup>2</sup> present. Sea water rinsing was effective for removing 84.3% of the microorganisms originally present.

However, as with the unfinished wooden sorting/heading table, a temporary (nine-fold) increase in TPC was experienced following scrubbing with laundry detergent. Again, this increase in microbial numbers was most likely due to hidden microbes being scrubbed out of nicks and scratches and floated to the surface by the surface active agents in the detergent. However, when chlorine bleach was applied, cooler surface TPC's were reduced by 98.8% (see Figure 3).

The results of 213 determinations for total coliforms (by the MPN three-tube technique) revealed their occurrence ( $>1$  organism/cm<sup>2</sup>) in less than 10% of all swabs taken from the three shrimp boat product handling and storage surfaces (see Tables 1-3). Thus, it seemed apparent that (total) coliform contamination was not a major problem associated with such on-board surfaces. However, it should be noted that the majority of the positive tests for coliforms occurred in swab samples taken from storage cooler surfaces. This phenomenon was consistent with the general microbiological observation that coliforms, which require a minimum water activity ( $a_w$ ) of 0.96, are quite labile to dessication (6). In fishing, therefore, wind- and sun-drying appeared to be sufficient to destroy most of the coliforms on exposed handling surfaces. However, the higher frequency of positive MPN's from cooler surfaces indicated that sufficient drying did not occur to effectively destroy all (total) coliforms within the coolers.

The same trend was noted for fecal coliforms. With only three exceptions discovered during the course of this investigation, the results of 213 determinations for fecal coliforms revealed their minimal occurrence on all three shrimp boat product handling and storage surfaces sampled (see Tables 1-3). As was the case with the total coliform determinations, most positive MPN's for fecal coliforms occurred in swab samples taken from the storage cooler surfaces.

After enumerating the surface microbial loads present following routine product handling and the reductions of surface microbial numbers effected by the three experimental clean-up or disinfection procedures, the next portion of the study related surface microbial quality to the storage stability of shrimp held on ice (0° C) for 15 days.

Most of the cooperating commercial boat's shrimping activities during the sampling period were performed on sandy bottoms. Shrimp which were aseptically removed from the nets prior to any human contact (with sterilized stainless steel tongs) were found to contain an

average TPC of 2,287 organisms/gm. With one exception (4 org/gm), all MPN's for total coliforms were negative (<3 org/gm), and all MPN's for fecal coliforms were negative (<3 org/gm). Therefore, "natural" contamination of shrimp with either total coliforms or fecal coliforms from sea water or bottom matter appeared to be negligible.

Shrimp which contacted the three handling and storage surfaces cleaned by Method 1 had an average TPC of 20,300 organisms/gm by the end of the first day. Following contact with handling and storage surfaces cleaned by Method 2, an even larger average TPC of 56,200 organisms/gm was noted at the end of the day. However, when all product handling and storage surfaces were cleaned and sanitized by Method 3, and the shrimp were handled by workers whose hands had been sanitized ("Tamed Iodine" Scrub, West Chemical Products, Inc., New York, NY), an average TPC of only 1,078 organisms/gm was realized at the end of the same one-day period (see Figure 4).

Thus, through a combination of well sanitized boat surfaces, properly washed and sanitized hands, expeditious handling, and thorough washing, the shrimp began their storage period with fewer microorganisms present (by the TPC method) than did shrimp handled on boat surfaces cleaned by Methods 1 or 2. These findings bear out John Williams' recommendations to, "Keep it clean, keep it cool, and keep it moving," (11).

Shrimp which contacted handling and storage surfaces cleaned by Method 1 began the 15-day iced storage period with an average TPC which was slightly over 20,000 organisms/gm. However, the iced storage conditions reduced the average TPC by 54.2% on Day 3. The average TPC of these shrimp slowly but steadily increased until the beginning (Day 1) TPC of 20,000 organisms/gm was once again attained on Day 9. From this point, the average TPC jumped to 363,000 organisms/gm on Day 12, and remained at approximately the same count for the remainder of the experimental storage period (see Figures 5 and 8). At no time during the experiment did the TPC's of shrimp from this lot exceed the Georgia Department of Agriculture laboratory bacteria guideline for raw shrimp ( $1.0 \times 10^6$  organisms/gm) (7).

Shrimp which contacted handling and storage surfaces cleaned by Method 2 began the experimental storage period with an average TPC of 56,200 organisms/gm. However, iced storage conditions effectively reduced the relatively high initial TPC of this sample lot to 6,250 organisms/gm by Day 3. The average TPC of these shrimp slowly increased until an average of 18,200 organisms/gm was attained on



Day 9. From this point, the average TPC rose to 178,000 organisms/gm on Day 12, and by Day 15, these stored shrimp had an average TPC of  $6.77 \times 10^7$  organisms/gm (see Figures 6 and 8). As indicated by the curves plotted in Figures 6 and 8, shrimp samples from this lot exceeded the Georgia Department of Agriculture laboratory bacteria guideline for raw shrimp during Day 13 of the storage experiment.

Finally those shrimp which contacted handling and storage surfaces sanitized by Method 3 and were handled by workers whose hands had been sanitized began the experimental iced storage period with an average TPC of only 1,078 organisms/gm. Following three days of storage, the average TPC was further reduced to 668 organisms/gm. From this point, however, the shrimp TPC's exhibited a marked increase, and by Day 9, 25,500 organisms/gm were present. Continuing their exponential growth, the average TPC of shrimp in this lot reached  $1.07 \times 10^6$  on Day 12 (see Figures 7 and 8). This TPC was slightly in excess of the Georgia Department of Agriculture laboratory bacteria guideline for raw shrimp (7).

Tables 4 through 6 list total coliform and fecal coliform data for shrimp samples taken from the iced storage experiment. Throughout the 15-day period, shrimp which contacted handling and storage surfaces cleaned by Method 1 exhibited no substantial reductions in total coliform MPN's. Although a downward trend in MPN numbers could be noted during successive samplings, no negative MPN tests were noted after the third day of iced storage (see Table 4).

However, shrimp which contacted handling and storage surfaces cleaned by Method 2 showed a progressive reduction in total coliform numbers from sampling to sampling. Further, negative MPN's were noted from Day 1 through Day 15 of the study, and the frequency of negative MPN tests was greater when compared to the frequency of positive MPN tests (see Table 5).

Shrimp which contacted handling and storage surfaces sanitized by Method 3 and were handled by workers whose hands had been sanitized exhibited, with few exceptions, relatively constant total coliform MPN's throughout the 15-day storage period. Again, however, negative MPN's were noted from Day 1 through Day 15, and the frequency of negative MPN tests was greater when compared to the frequency of positive MPN tests (see Table 6).

Finally, no positive MPN tests for fecal coliforms were found among the 15-day storage study shrimp samples. This indicated that contamination with human (fecal) pathogens was not identified as a problem associated with handling of shrimp during the study.

Statistical analysis (one way analysis of variance) of TPC data from the 15-day storage stability experiment revealed the following information about the shrimp samples:

- 1.) At the 0.975 level of confidence, shrimp which contacted surfaces sanitized by Method 3 had significantly lower TPC's from Day 1 through Day 9 than did shrimp which contacted surfaces cleaned by either Method 1 or 2;
- 2.) At the 0.95 level of confidence, no significant difference was noted between TPC's for shrimp which contacted surfaces cleaned or sanitized by Methods 1 or 3 from Day 10 through Day 15; and,
- 3.) At the 0.95 level of confidence, TPC's for shrimp which contacted surfaces cleaned by Method 2 were significantly greater than were TPC's for shrimp which contacted surfaces cleaned or sanitized by Methods 1 or 3 from Day 10 through Day 15.

#### CONCLUSIONS

By quickly handling the catch on cleanly-maintained surfaces by workers with sanitized hands, this study illustrated that:

- 1.) The TPC's of shrimp handled on surfaces sanitized by Method 3 were substantially lower than were the TPC's of shrimp handled on surfaces cleaned by either Method 1 or Method 2;
- 2.) Significant reductions in the TPC's of shrimp which contacted surfaces sanitized by Method 3 were effected through the ninth day of iced storage; and,
- 3.) Contamination by human handling (as noted by tests for fecal coliforms and total coliforms) was noted in less than 10% of all determinations.

In essence, this study provided a basis of proof to illustrate the point that wooden boat surfaces, whether sealed with fiberglass or not, can be cleaned very well. Based upon the statistical analysis of the storage study, however, it seemed apparent that Clean-up Methods 2 and 3 did not lengthen the storage life of the shrimp beyond the life provided by Clean-up Method 1.

## ACKNOWLEDGEMENTS

The technical assistance of Ms. Lea Dowdy and Ms. Beverly Lea is gratefully acknowledged.

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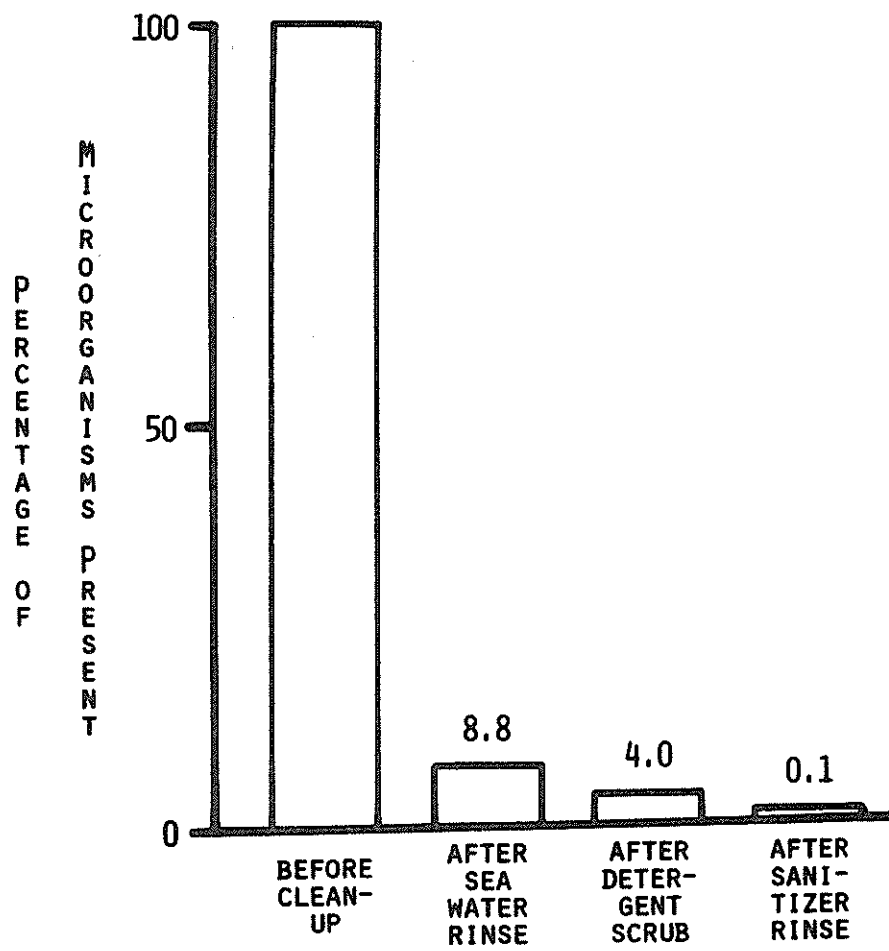


Figure 1. Effectiveness of Sanitization -  
Fiberglassed Wooden Deck.

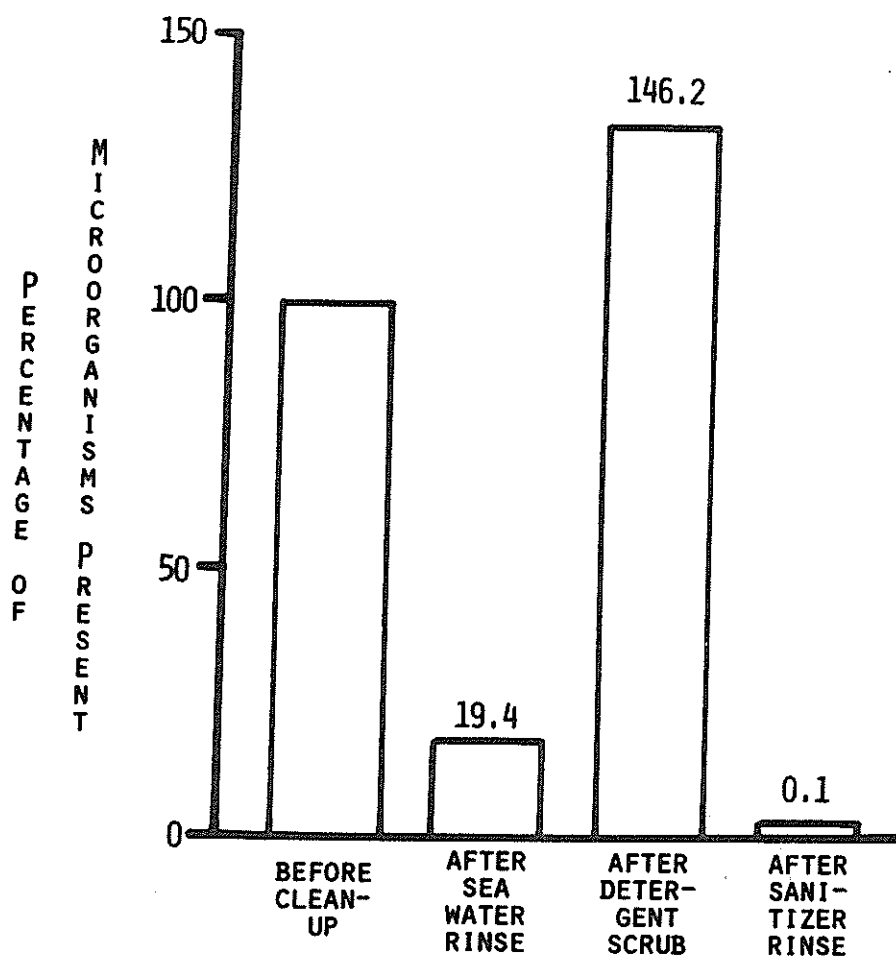


Figure 2. Effectiveness of Sanitization - Unfinished Wooden Sorting Table.

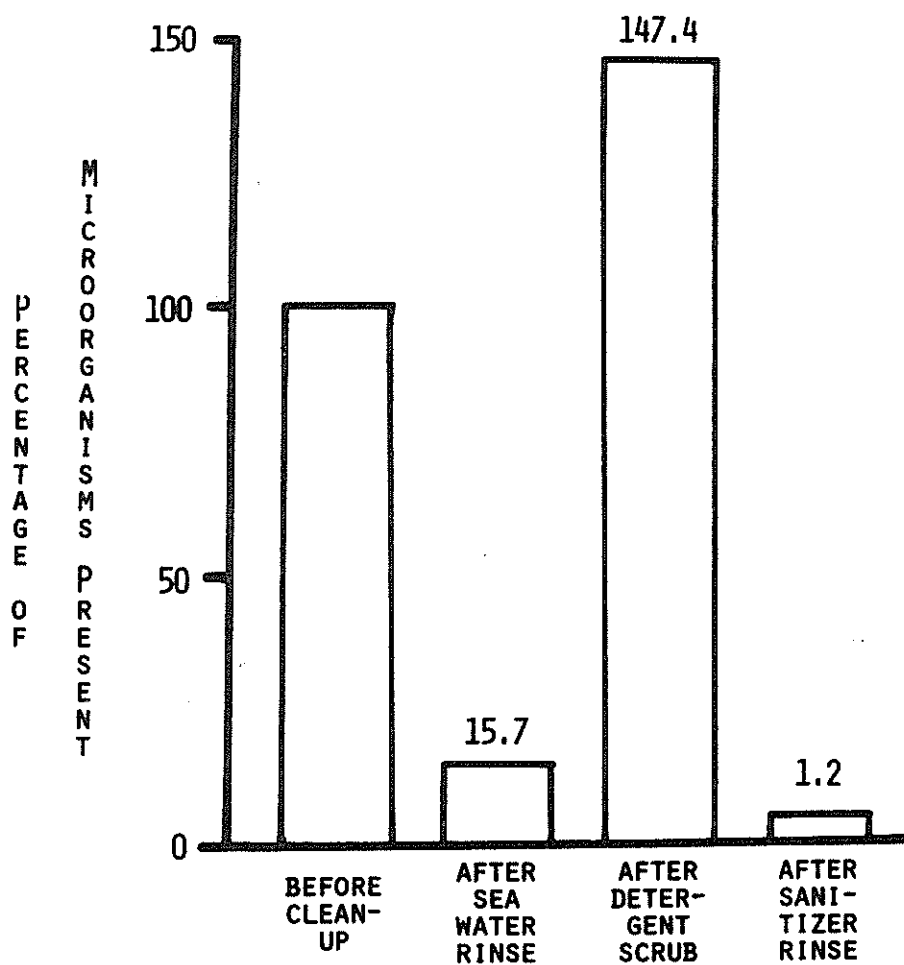


Figure 3. Effectiveness of Sanitization - Plastic Storage Coolers.

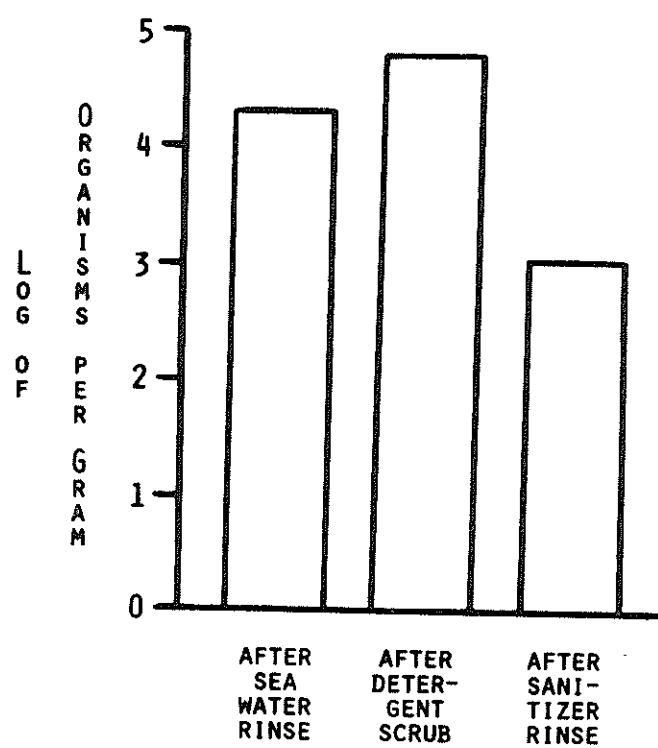


Figure 4. Effectiveness of Sanitization - Landed Shrimp.

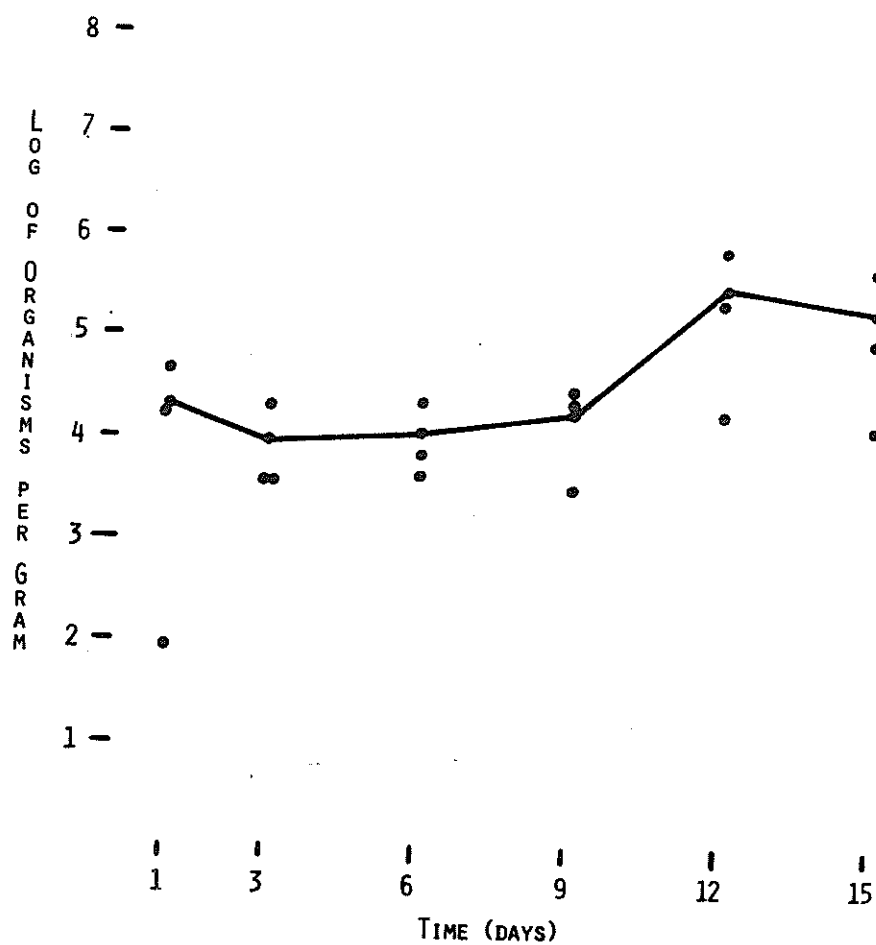


Figure 5. Iced Shrimp Storage -  
After Sea Water Rinse.



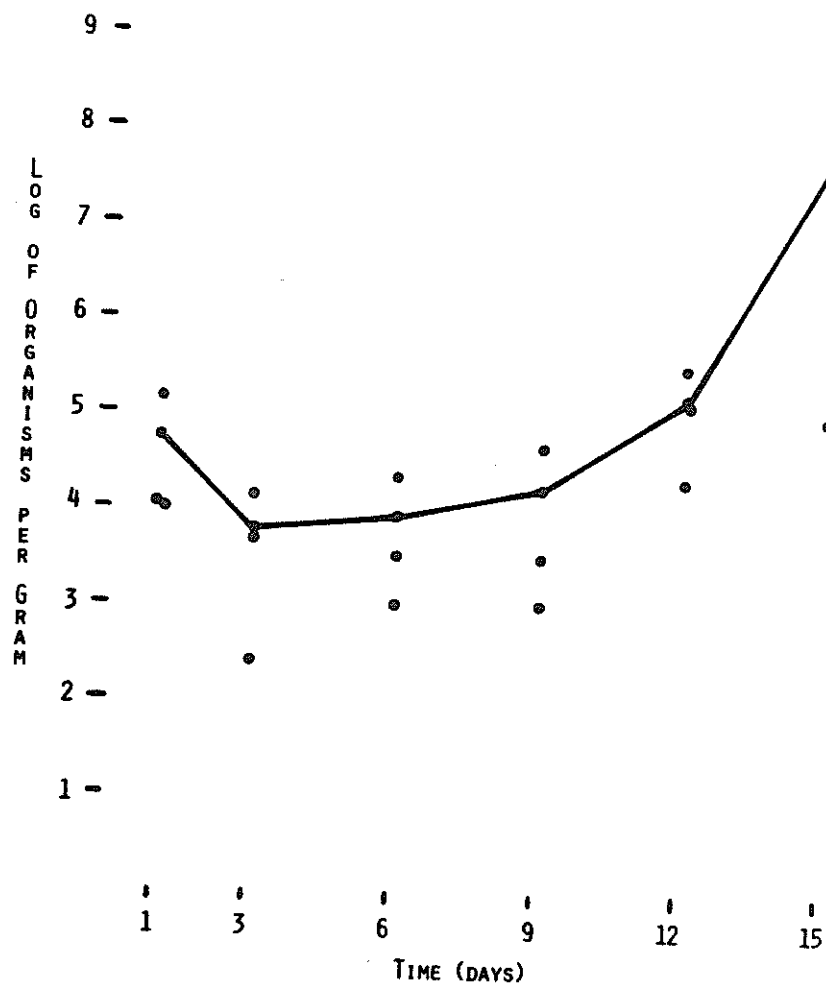


Figure 6. Iced Shrimp Storage -  
After Detergent Scrub.

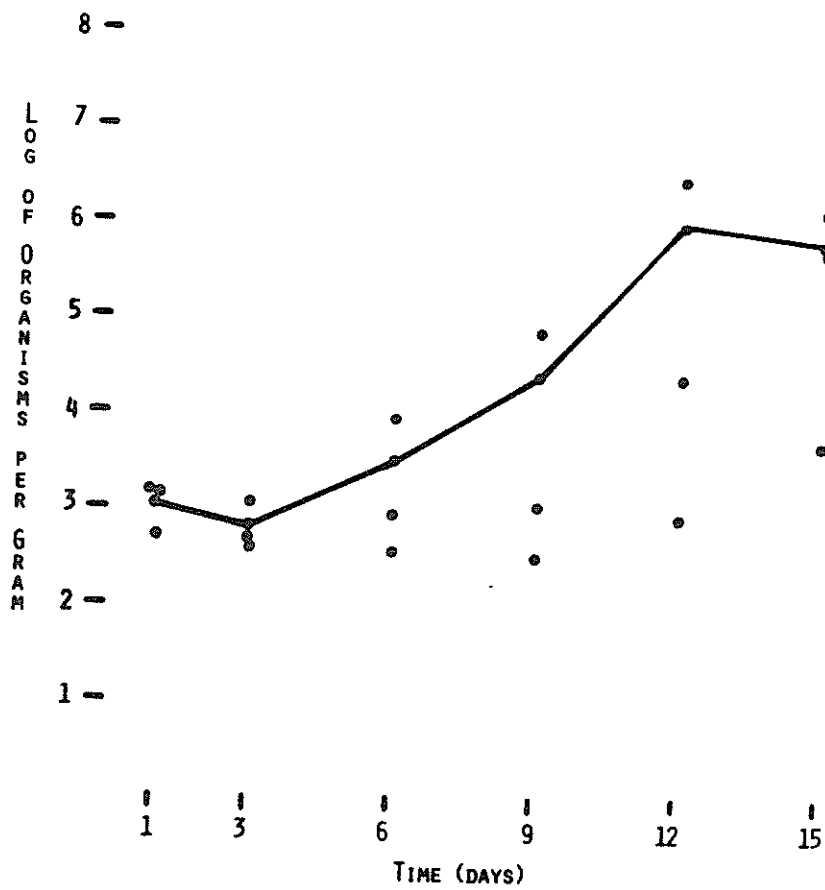


Figure 7. Iced Shrimp Storage -  
After Sanitizer Scrub.

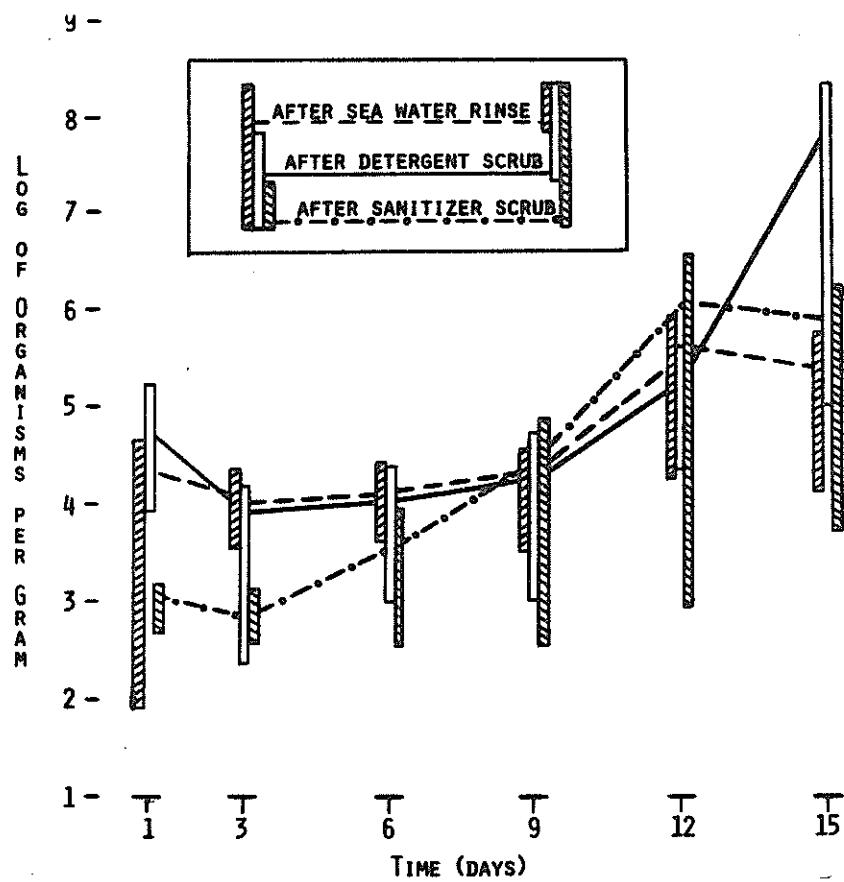


Figure 8. Iced Shrimp Storage - Composite.

	Coliforms/cm <sup>2</sup>		Fecal Coliforms/cm <sup>2</sup>	
	Freq.	MPN	Freq.	MPN
<u>Sea Water Rinse</u>				
Before Fishing	6	<1	8	<1
	2	4	1	10
	1	6		
After Fishing	7	<1	9	<1
	2	1		
After Clean-Up	9	<1	9	1
<u>Detergent Scrub</u>				
Before Fishing	9	<1	9	<1
After Fishing	9	<1	9	<1
After Clean-Up	9	<1	9	<1
<u>Sanitizer Scrub</u>				
Before Fishing	9	<1	9	<1
After Fishing	8	<1	9	<1
	1	1		
After Clean-Up	9	<1	9	<1

Table 1. Total Coliforms and Fecal Coliforms -  
Fiberglassed Wooden Deck.

	Coliforms/cm <sup>2</sup>		Fecal Coliforms/cm <sup>2</sup>	
	Freq.	MPN	Freq.	MPN
<u>Sea Water Rinse</u>				
Before Fishing	9	<1	9	<1
After Fishing	7	<1	9	<1
	1	2		
	1	1		
After Clean-Up	9	<1	9	<1
<u>Detergent Scrub</u>				
Before Fishing	9	<1	9	<1
After Fishing	9	<1	9	<1
After Clean-Up	9	<1	9	<1
<u>Sanitizer Scrub</u>				
Before Fishing	9	<1	9	<1
After Fishing	9	<1	9	<1
After Clean-UP	9	<1	9	<1

Table 2. Total Coliforms and Fecal Coliforms -  
Unfinished Wooden Sorting Table.

	Coliforms/cm <sup>2</sup>		Fecal Coliforms/cm <sup>2</sup>	
	Freq.	MPN	Freq.	MPN
<u>Sea Water Rinse</u>				
Before Fishing	7	<1	8	<1
	1	8	1	1
	1	2		
After Fishing	8	<1	9	<1
	1	18		
<u>Detergent Scrub</u>				
Before Fishing	6	<1		
	1	≤96	8	<1
	1	18	1	4
	1	4		
After Fishing	2	<1	6	<1
	2	2		
	1	≤96		
	1	44		
<u>Sanitizer Scrub</u>				
Before Fishing	9	<1	9	<1
After Fishing	7	<1	9	<1
	2	1		

Table 3. Total Coliforms and Fecal Coliforms -  
Plastic Storage Coolers

<u>Day of Storage</u>	<u>Coliforms/gm</u>		<u>Fecal Coliforms/gm</u>	
	<u>Freq.</u>	<u>MPN</u>	<u>Freq.</u>	<u>MPN</u>
1	1	1100	3	<3
	1	23		
	1	<3		
3	1	240	3	<3
	1	4		
	1	<3		
6	2	7	3	<3
	1	240		
9	1	93	3	<3
	1	43		
	1	9		
12	2	4	3	<3
	1	75		
15	1	23	3	<3
	1	9		
	1	4		

Table 4. Iced Shrimp Storage -  
Total Coliforms and Fecal Coliforms  
(After Sea Water Rinse).

<u>Day of Storage</u>	<u>Coliforms/gm</u>		<u>Fecal Coliforms/gm</u>	
	<u>Freq.</u>	<u>MPN</u>	<u>Freq.</u>	<u>MPN</u>
1	2 1	<3 23	3	<3
3	2 1	<3 9	3	<3
6	3	<3	3	<3
9	3	<3	3	<3
12	2 1	<3 4	3	<3
15	3	<3	3	<3

Table 5. Iced Shrimp Storage -  
Total Coliforms and Fecal Coliforms  
(After Detergent Scrub).



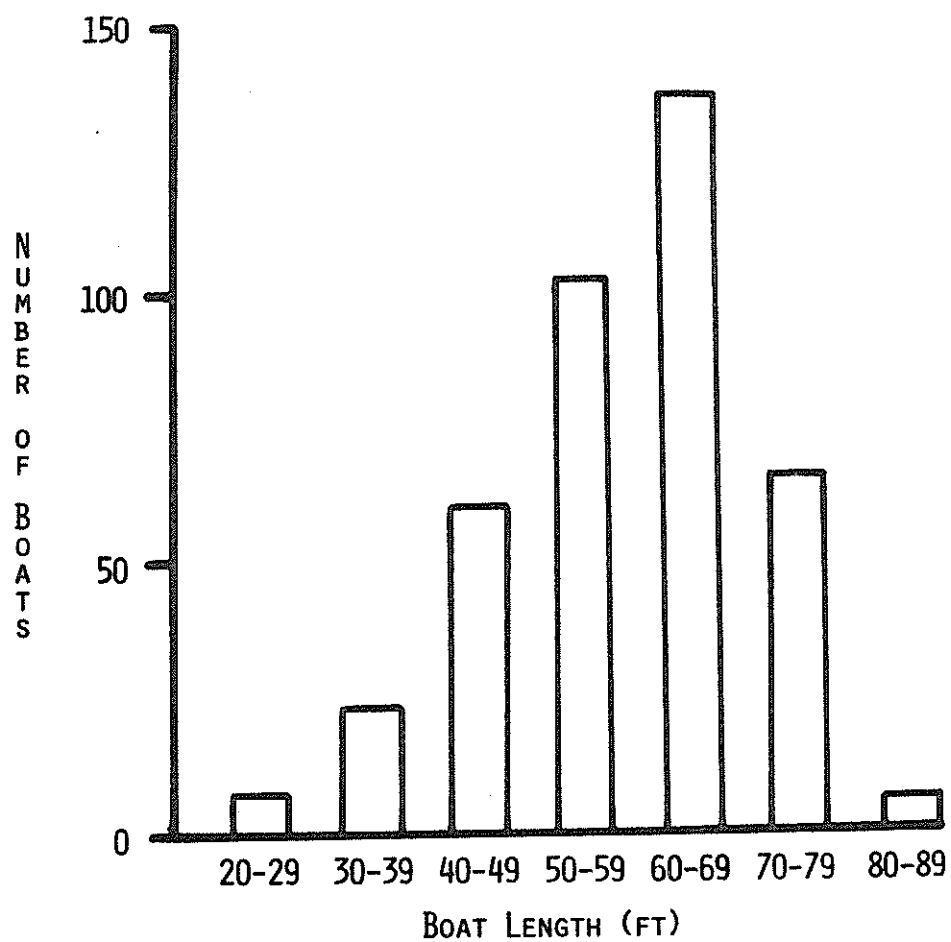
Day of Storage	Coliforms/gm		Fecal Coliforms/gm	
	Freq.	MPN	Freq.	MPN
1	2	<3	3	<3
	1	4		
3	2	<3	3	<3
	1	23		
6	2	<3	3	<3
	1	43		
9	2	<3	3	<3
	1	21		
12	2	<3	3	<3
	1	23		
15	1	4	3	<3
	1	3		
	1	<3		

Table 6. Iced Shrimp Storage -  
Total Coliforms and Fecal Coliforms  
(After Sanitizer Scrub).

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APPENDIX  
(Shrimp Boat Sanitation)



Appendix Figure 1. Georgia Vessels Licensed for Commercial Shrimping During the 1978 Season.

Number of Vessels	Vessel Construction
12	Steel
53	Fiberglass
333	Wood
Total 398	

\*Georgia Department of Natural Resources, Coastal  
Resources Division: 1978 Commercial Shrimping Licenses.

Appendix Table 1. Construction of Georgia Vessels  
Licensed for Commercial Shrimping  
During the 1978 Season\*.

FREE LIQUID CONTENT OF OYSTERS HARVESTED FROM  
APALACHICOLA BAY DURING THE MONTH OF MARCH, 1978

Billy G. Miles, Food Laboratory  
Florida Department of Agriculture and Consumer Services

INTRODUCTION: How much do oysters bleed? This has been a controversial question for many years. Oyster dealers feel that they are being unfairly prosecuted by states that have a free liquid tolerance for oysters and consumer complaints about packs of oysters that contain 50% free liquid.

The Florida Department of Agriculture, wanting to be fair to all parties concerned, decided to reevaluate its tolerance of no more than 15% free liquid when the oysters are purchased by the consumer.

The plan of action was to put up packs of oysters in accordance with the Code of Federal Regulations, Title 21. This study was conducted in November, 1976, and January, 1977, and showed that if oysters are packed that way, they would comply with the state of Florida free liquid requirements.

After reviewing the results from the study, it was decided that additional data was needed to determine when oysters bled the most.

Representatives of the Florida Department of Agriculture and Consumer Services meet with representatives of the oyster industry from the Apalachicola area and asked them what month was the worse month for oysters to bleed. They all state that March is the worse month.

Arrangements were made with the Inspection Division and six oyster processors in Apalachicola to put up, once a week for the month of March, oysters for this study. The oysters were packed in accordance with the adopted federal requirements in the Code of Federal Regulations, Title 21. Also, the area where the oysters were harvested, wind direction, and weather was recorded.

#### EXPERIMENT

Oysters were packed on March 1, 8, 15, 22, 29 and April 11, 1978. The oysters were shucked by regular shuckers and samples were taken by random collection of oysters brought into the processing room. The oysters were washed and packed into 12 oz., 15½ oz. or 16 oz. containers, depending on the size container the firm normally processed. Portions of the packs were opened at the plants and free liquid content was determined. The remainder of

the packs were stored in ice chests covered with ice, and transported by car to the laboratory in Tallahassee. When the oysters were received by the laboratory, they were stored in ice chests covered with ice, in a walk-in refrigerator at 40°F until analyses were completed.

Determinations were conducted six days after the day of pack. Each container was drained for two minutes on an eight inch diameter, 3/8 inch opening sieve. After draining, free liquid was measured and percent free liquid was calculated. An additional container from each processor was examined microbiologically for APC at 37°C, coliforms, E. coli, coagulase positive staphylococci and Salmonella (AOAC).

## RESULTS AND DISCUSSION

Tables 1 - 6 list plant, date of pack, number samples analyzed at plant, average percent free liquid, date analyzed by laboratory, average percent free liquid, area harvested (east or west end of bay), wind direction and weather. Table 7 is a summary of tables 1 - 6. The average percentage free liquid ranged from 1.70% on 3-1-78, to 21.78% on 4-4-78, and back down to 5.88% on 4-11-78. The minimum percent free liquid was 0.5% and the maximum percent free liquid was 28.2%. The area where harvested and direction of the wind appeared to have little influence and the amount oysters bleed, but the amount of rain affected the salinity of the water and caused the oysters to absorb water which causes them to bleed when processed and increase the percent free liquid.

Tables 8 - 12 list the total bacterial counts at 37°C/gm, coliforms, E. coli, coagulase positive staphylococci MPN and Salmonella/gm, over the period of 3-2-78 to 3-29-78. The study indicates that most of the oysters were not in compliance with the bacteriological tolerances of the National Shellfish Certification Program. Standard plate count ranged from 53,000/gm to 4,000,000/gm, coliforms ranged from 15/gm to 11,000/gm, E. coli ranged from 0 to 460 MPN, and one sample contained coagulase positive staphylococci, 3.6 MPN and no samples contained Salmonella.

## SUMMARY

Analyses revealed that some oysters packed in accordance with the Code of Federal Regulations Title 21 will bleed up to 28.2% after a week of storage on ice, but the majority of the time, the oysters would bleed less than 15% after a week of storage on ice. Also, the increase in viable bacteria could contribute to the increase in percent free liquid. The increase in viable bacteria was probably due to run off from the rain and the fact that the U. S. Corp of Engineers was dredging a channel into East Point at the time oysters were harvested. A more intensified study over a five year period is needed before any prediction of how much an Apalachicola oyster will bleed can be made.

TABLE 1

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>
A	3-1-78	4	1.9%
B	3-1-78	4	1.6%
C	3-1-78	4	3.6%
D	3-2-78	4	2.8%
E	3-2-78	4	3.4%
F	3-2-78	4	3.3%
AVERAGE			2.8%

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>	<u>Area of Harvest</u>	<u>Weather Rain</u>	<u>Wind</u>
A	3-7-78	4	0.85%	West	None	Variable
B	3-7-78	4	1.41%	East	None	Variable
C	3-7-78	4	1.74%	West	None	Variable
D	3-7-78	4	2.37%	West	None	Variable
E	3-7-78	3	1.63%	East	None	Variable
F	3-7-78	4	2.07%	East	None	Variable
AVERAGE			1.7%			

All samples examined on 3-7-78 did not exceed 15% free liquid requirements.

Range 0.5 - 2.5%



TABLE 2

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>
A	3-8-78	4	2.60%
B	3-8-78	3	1.67%
C	3-8-78	4	3.17%
D	3-8-78	4	3.17%
E	3-8-78	3	4.01%
F	3-8-78	4	3.80%
AVERAGE			3.07%

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>	<u>Area of Harvest</u>	<u>Weather Rain</u>	<u>Wind</u>
A	3-14-78	4	7.46%	West	2.21"	S.E.
B	3-14-78	3	7.72%	West	2.21"	S.E.
C	3-14-78	4	5.27%	West	2.21"	S.E.
D	3-14-78	4	8.85%	East	2.21"	S.E.
E	3-14-78	3	7.22%	West	2.21"	S.E.
F	3-14-78	4	7.08%	East	2.21"	S.E.
AVERAGE			7.26%			

All samples examined on 3-14-78 do not exceed 15% free liquid requirements.

Range 4.51 - 9.41%

TABLE 3

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>
A	3-15-78	4	2.53%
B	3-15-78	4	1.78%
C	3-15-78	4	2.96%
D	3-15-78	4	3.94%
E	3-15-78	3	2.85%
F	3-15-78	4	3.31%
AVERAGE			3.9%

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>	<u>Area of Harvest</u>	<u>Weather Rain</u>	<u>Wind</u>
A	3-21-78	4	10.95%	West	Trace	Variable
B	3-21-78	3	20.53%*	East	Trace	Variable
C	3-21-78	4	7.9%	West	Trace	Variable
D	3-21-78	4	21.9%*	East	Trace	Variable
E	3-21-78	4	6.9%	East	Trace	Variable
F	3-21-78	4	13.51%	West	Trace	Variable
AVERAGE			13.62%			

9 samples examined on 3-21-78 exceeded the 15% free liquid requirement.

Range 5.06 - 28.2%

TABLE 4

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>
A	3-22-78	4	2.60%
B	3-22-78	3	2.18%
C	3-22-78	4	2.11%
D	3-22-78	4	3.32%
E			
F	3-22-78	4	4.08%
AVERAGE			2.86%

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>	<u>Area of Harvest</u>	<u>Weather Rain</u>	<u>Wind</u>
A	3-28-78	4	6.14%	West	None	S.W.
B	3-28-78	4	7.5%	West	None	S.W.
C	3-28-78	4	4.01%	West	None	S.W.
D	3-28-78	4	22.74%*	West	None	S.W.
E						
F	3-28-78	4	12.6%	East	None	S.W.
AVERAGE			10.60%			

4 samples examined on 3-28-78 exceeded the 15% free liquid requirement.

Range 2.81 - 25.07%

TABLE 5

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>
A	3-29-78	4	3.17%
B	3-29-78	3	0.96%
C	3-29-78	4	2.25%
D	3-29-78	3	6.06%
E			
F	3-29-78	4	1.48%
AVERAGE			3.48%

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>	<u>Area of Harvest</u>	<u>Weather</u> <u>Rain</u>	<u>Wind</u>
A	4-4-78	4	25.00%*	East	None	S.W.
B	4-4-78	4	18.32%*	East	None	S.W.
C	4-4-78	4	17.46%	East	None	S.W.
D	4-4-78	4	26.13%*	West	None	S.W.
E						
F	4-4-78	4	22.02%*	East	None	S.W.
AVERAGE			21.78%			

All but one sample examined on 4-4-78 exceeded the 15% free liquid requirements.

Range 14.08 - 26.48%

TABLE 6

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>
B	4-11-78	3	0.33%
D	4-11-78	4	0.28%
AVERAGE			0.30%

<u>Plant</u>	<u>Date</u>	<u># Samples Examined</u>	<u>Average % Free Liquid</u>	<u>Area of Harvest</u>	<u>Weather</u>	
					<u>Rain</u>	<u>Wind</u>
B	4-18-78	3	6.77%	East	None	S.W.
D	4-18-78	4	5.00%	West	None	S.W.
AVERAGE			5.88%			

All samples examined 4-18-78 did not exceed 15% free liquid requirement.

Range 3.9 - 7.4%

TABLE 7  
S U M M A R Y

Date of Collection	3-1-78	3-8-78	3-15-78	3-22-78	3-29-78	4-11-78
# Samples Analyzed on Date of Collection	24	23	23	19	18	7
Average % Free Liquid	2.8%	3.07%	2.9%	2.8%	3.48%	0.30%
Date	3-7-78	3-14-78	3-21-78	3-28-78	4-4-78	4-18-79
# Samples Examined	23	22	23	20	20	7
Average % Free Liquid	1.7%	7.26%	13.62%	10.60%	21.78%	5.88%
Range % Free Liquid	0.5-2.5	4.51-9.41	5.04-28.2	2.81-25.07	14.08-26-48	3.9-7.4
Area of Harvest East/West	50%/50%	33%/66%	50%/50%	20%/80%	80%/20%	50%/50%
Weather/Rain	none	2.2"	Trace	None	None	None
Weather/Wind	Variable	S.E.(20*)	Variable	S.W.(14*)	S.W.(11*)	S.W.(14*)

\* miles per hour

TABLE 8

PLANT A

<u>Date</u>	<u>SPC bacteria/gm</u>	<u>Coliforms</u>	<u>E. coli</u>	<u>Coag + Staph</u>	<u>Salmonella</u>
3-2-78	630,000	430	neg	neg	neg
3-9-78	53,000	23	neg	neg	neg
3-17-78	630,000	≥1100	neg	neg	neg
3-23-78	1,000,000	36	460	neg	neg
3-29-78	630,000	1100	neg	neg	neg

TABLE 9

PLANT B

<u>Date</u>	<u>SPC bacteria/gm</u>	<u>Coliforms</u>	<u>E. coli</u>	<u>Coag + Staph</u>	<u>Salmonella</u>
3-2-78	600,000	930	neg	neg	neg
3-9-78	170,000	1100	43	neg	neg
3-17-78	>3,000,000	>1100	neg	neg	neg
3-23-78	4,000,000	>1100	neg	neg	neg
3-29-78	540,000	>1100	neg	neg	neg

TABLE 10

PLANT C

<u>Date</u>	<u>SPC bacteria/gm</u>	<u>Coliforms</u>	<u>E. coli</u>	<u>Coag + Staph</u>	<u>Salmonella</u>
3-2-78	93,000	230	neg	neg	neg
3-9-78	240,000	43	neg	neg	neg
3-17-78	2,500,000	1100	3.6	neg	neg
3-23-78	2,400,000	1100	460	neg	neg
3-30-79	430,000	460	neg	neg	neg

TABLE 11

PLANT D

<u>Date</u>	<u>SPC bacteria/gm</u>	<u>Coliforms</u>	<u>E. coli</u>	<u>Coag + Staph</u>	<u>Salmonella</u>
3-3-78	230,000	110	neg	neg	neg
3-9-78	500,000	460	neg	neg	neg
3-17-78	2,000,000	1100	neg	3.6	neg
3-23-78	>3,000,000	1100	neg	neg	neg
3-29-78	1,400,000	1100	neg	neg	neg



TABLE 12

PLANT E

<u>Date</u>	<u>SPC bacteria/gm</u>	<u>Coliforms</u>	<u>E. coli</u>	<u>Coag + Staph</u>	<u>Salmonella</u>
3-3-78	420,000	230	neg	neg	neg
3-9-78	280,000	150	neg	neg	neg
3-17-78	1,300,000	210	neg	neg	neg

TABLE 13

PLANT F

<u>Date</u>	<u>SPC bacteria/gm</u>	<u>Coliforms</u>	<u>E. coli</u>	<u>Coag + Staph</u>	<u>Salmonella</u>
3-3-78	110,000	91	neg	neg	neg
3-9-78	64,000	160	neg	neg	neg
3-17-78	3,300,000	≥1100	3.6	neg	neg
3-23-78	>3,000,000	>1100	3.0	neg	neg
3-29-78	680,000	15	neg	neg	neg

## BACTERIOLOGICAL SURVEY OF ICED COMMERCIAL OYSTERS AT THE RETAIL LEVEL

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Oysters are by far the leading commercial fishery product among the marine mollusks. They are also ranked high among all fishery products in dollar value at harvest. Approximately 40 million pounds of oyster meat are produced in the United States, primarily of the Eastern oyster, Crassostrea virginica. The Eastern oyster is widely distributed in the coastal waters and bays of the United States from Massachusetts to Texas.

Oysters are non-motile organisms dependent on a filtering of water through the gills to obtain food. As a consequence of this filter feeding action, microorganisms and chemicals including toxic metal can be accumulated from the marine environment. The quality of oysters is therefore related closely to the environment in which they grow and feed.

A major problem in the commercial production of oysters is the prevention of microbial contamination. Two prime factors that account for such contamination are: (1) the microbial pollution of oyster beds and (2) subsequent contamination by handling during processing.

The purpose of this investigation was to determine the sanitary quality of oysters as reflected by their microbial profile utilizing the following: (1) the Aerobic Plate Count at 35°C, (2) the Most Probable Number of total coliforms, (3) the Most Probable Number of Escherichia coli, and (4) the Most Probable Number of coagulase-positive staphylococci.

### MATERIALS AND METHODS

Oyster samples which had been shucked, washed with potable water and packed in glass containers or cardboard boxes were purchased from sixteen different retail outlets in the Baton Rouge area. Controls for the study were obtained in screw cap pint glass jars from a certified and U.S.F.D.A. approved wholesale distributor in New Orleans.

Retail samples were packed in ten and twelve ounce glass or cardboard containers and controls were packed in screw cap pint jars.

The retail samples were transported in ice from their respective outlets to the Food Science Department at Louisiana State University within a one hour period. Control samples were treated identically and arrived at the Food Science Department within a two hour period. Upon arrival at Louisiana State University, all samples and controls were placed in fresh ice for subsequent microbial analyses.

This study was conducted over a six month period extending from August 1977 to February 1978. Analyses were simultaneously run on a monthly basis on samples from ten to sixteen participating retail outlets. Controls were routinely analyzed at the same time as the retail samples.

Analyses of samples were conducted immediately upon arrival at the Department of Food Science Laboratory, L.S.U., Baton Rouge, La. and after one week storage on ice. All analyses were conducted in duplicate.

The oyster homogenate was prepared by aseptically weighing 50 g of oyster meat from the glass container into a tared sterile petri dish. Immediately, the samples were transferred into sterile Waring Blendor Jars, and 450 ml of Butterfield's phosphate buffer water were added. The samples were homogenized for 60 to 120 seconds in the Waring Blendor at low speed (8,000-10,000 rpm). The 1:10 oyster homogenate was then allowed to stand at room temperature for 5 minutes until phase separation occurred. Serial dilutions were made from the 1:10 oyster homogenate by aseptically transferring 10 ml aliquots into 90 ml of sterile Butterfield's phosphate buffer dilution blanks. This initial dilution and subsequent dilutions were used to determine Aerobic Plate Counts (APC), Total Coliforms, (TC), *E. coli* and Coagulase Positive *Staphylococcus aureus* according to the Bacteriological Analytical Manual for Foods.

## RESULTS AND DISCUSSION

### The Aerobic Plate Count

The Aerobic Plate Count (A.P.C.) values of the 65 samples of oysters as purchased range from  $3.5 \times 10^3$  to  $\geq 30 \times 10^6$  organisms/g as seen in Table I. The mean of the 65 samples over the 6 month period, based on duplicate determinations for each of the samples, was  $2.4 \times 10^6$ .

After the samples of oysters had been stored on ice for seven days, the A.P.C. values ranged from  $20 \times 10^3$  to  $\geq 30 \times 10^6$  organisms/g. The mean value for the six month observations for stored oysters was  $12.3 \times 10^6$ . This corresponded to an increase of more than five fold during the 7-days storage period.

The monthly mean A.P.C. values for oysters as purchased were highest in December and lowest in November. Low values, only 5 to 9% greater than the November mean, also were associated with the oysters purchased in September, October and January.

After the oysters had been stored seven days on ice, the monthly mean A.P.C. values had increased many fold. These values were highest in December and lowest in August. The same monthly relationships also prevailed in the overall six month mean value.

#### Most Probable Number of Total Coliforms

Among the 65 samples of oysters purchased at retail outlets, the M.P.N. for total coliforms/100g ranged from 400 to  $\geq 24 \times 10^4$ . The overall six month mean, based on duplicate determinations for each sample, was  $13.9 \times 10^4$  M.P.N. of total coliforms/100 g.

After being stored for 7 days on ice, the range of total coliform/100g M.P.N. was still  $4 \times 10^2$  to  $\geq 24 \times 10^4$  but the overall mean value for the 65 samples had increased to  $17.3 \times 10^4$ . In relative terms, the numbers of total coliforms increased 25% during the storage period. The monthly mean values for oysters as purchased were lowest in September and highest in January with a five-fold difference separating the two extremes which were  $4.2 \times 10^4$  and  $2.2 \times 10^4$ .

After the oysters had been stored on ice for seven days, the monthly mean total coliform/100g M.P.N. values ranged from  $13 \times 10^4$  for oysters purchased in August to almost  $23 \times 10^4$  for those purchased in January.

#### Most Probable Number of E. coli

The E. coli values of the 65 samples of oysters as purchased ranged from 0 to  $24 \times 10^4$  E. coli/100g M.P.N. Based on duplicate determinations for each sample, the overall six month mean was  $53 \times 10^2$  E. coli/100g M.P.N.

After the oysters had been stored on ice for seven days, the E. coli values were still in the range, 0 to  $\geq 24 \times 10^4$  M.P.N. of E. coli/100 g, but the overall six month mean had increased 67% to the higher value  $89 \times 10^2$  M.P.N. E. coli/100 g. The monthly mean value for E. coli in the samples of oysters as purchased were lowest in January and highest in October.

After the oysters had been stored seven days on ice, the monthly mean E. coli/100g M.P.N. values ranged from 730 for oysters purchased in August to  $290 \times 10^2$  for those purchased in October.

#### Most Probable Number of Coagulase-Positive S. aureus

Among the 65 samples of oysters as purchased at the retail outlets over a period of six months, the values for coagulase-positive S. aureus/g ranged from 0 to 2,400/g M.P.N. The overall six month

mean of the 65 samples, based on duplicate determinations for each sample, was 15.5/g coagulase-positive S. aureus M.P.N.

After being stored for seven days on ice, the range of the coagulase-positive S. aureus/g values was from 0 to  $\geq 2,400$ /g M.P.N. The overall six month mean value had increased 3 fold to 48.4/g coagulase positive S. aureus M.P.N. The monthly mean values for oysters as purchased were lowest in January and highest in November. The values ranged from 0.6 to 70.9/g M.P.N.

After the oysters had been stored on ice for seven days, the monthly mean coagulase-positive S. aureus values ranged from 4.3 for oysters purchased in September and January to 219.4 M.P.N.'s/g for those purchased in November.

The correlations between A.P.C. values and those of total coliforms, E. coli and Coagulase-positive S. aureus were positive and significant at the 1% level.

The correlation between the total coliform values and E. coli values was positive and just barely above the 5% level ( $0.052 - 0.050 = 0.002$ ).

The correlation between the total coliform values and Coagulase-positive S. aureus values was negative but it was not significant.

The correlation between the E. coli values and the coagulase-positive S. aureus values was negative but it was not significant.

#### CONCLUSIONS

The substantial bacterial variations among the different oyster samples from the retail outlets studied in this survey were probably a consequence of the poor handling practices employed in each location. These poor handling practices included the use of improper packaging materials, infected and insanitary employees, the direct handling of oyster meat, and overall improper handling and storage of the oysters are some of the variables which may account for these unreasonably high microbial populations. The introduction of handling procedures which conform to recommended federal good manufacturing practices (G.M.P.'s) and other recommendations should result in consumer products with lower bacterial levels thus providing the consumer the opportunity to purchase fresh, wholesome, safe and nutritious oysters.

#### REFERENCES

- U. S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE. 1979.  
Bacteriological analytical manual for foods. U. S. Food and Drug Administration, Washington, D. C.

Table 1. Monthly Mean A. P. C. Values\* For Oyster Purchased  
Over A Period Of Six Months

Month	N	Range <sub>3</sub> x 10 <sup>3</sup>	Mean <sub>6</sub> x 10 <sup>6</sup>
A.			
August	20	9.9 - 16,200	3.4
September	22	6.0 - 12,000	1.5
October	22	8.9 - 9,800	1.5
November	22	3.5 - 6,300 <sup>a</sup>	1.4
December	22	8.3 - 30,000 <sup>a</sup>	4.4
January	22	3.6 - 13,200	1.6
Mean for 6 Month Observations			2.4
B.			
August	20	20.0 - 19,000	7.3
September	22	186.0 - 30,000 <sup>a</sup>	10.4
October	22	1,280.0 - 30,000 <sup>a</sup>	11.8
November	22	1,780.0 - 30,000 <sup>a</sup>	13.7
December	22	410.0 - 30,000 <sup>a</sup>	16.8
January	22	1,640.0 - 30,000 <sup>a</sup>	13.2
Mean for 6 Month Observations			12.3

\* A. P. C. values expressed as Organisms/g.

<sup>a</sup> Quantity either equal or greater than listed.

A = As Purchased; B = After 7 Days Iced Storage;

N = Number of Observations

Table 2. Monthly Mean Total Coliforms Values\* For Oysters  
Purchased Over A Period Of Six Months

Month	N	Range $\times 10^2$	Mean <sub>4</sub> $\times 10^4$
A.			
August	20	4.0 - 2,400 <sup>a</sup>	15.5
September	22	4.0 - 2,400 <sup>a</sup>	4.2
October	22	4.0 - 2,400 <sup>a</sup>	10.0
November	22	23.0 - 2,400 <sup>a</sup>	13.8
December	22	7.0 - 2,400 <sup>a</sup>	18.4
January	22	15.0 - 2,400 <sup>a</sup>	21.9
Mean For 6 Month Observations			13.9
B.			
August	20	9.0 - 2,400 <sup>a</sup>	13.0
September	22	9.0 - 2,400 <sup>a</sup>	13.2
October	22	4.0 - 2,400 <sup>a</sup>	15.9
November	22	150.0 - 2,400 <sup>a</sup>	18.2
December	22	43.0 - 2,400 <sup>a</sup>	20.4
January	22	110.0 - 2,400 <sup>a</sup>	22.8
Mean For 6 Month Observations			17.3

\* Total coliforms values expressed as M.P.N. of total coliforms/100 g.

<sup>a</sup> Quantity either equal or greater than listed.

A = As Purchased; B = After 7 Days Iced Storage;  
N = Number of Observations

Table 3. Monthly Mean Escherichia coli Values\* For Oysters  
Purchased Over A Period Of Six Months

Month	N	Range x 10 <sup>2</sup>	Mean <sub>2</sub> x 10 <sup>2</sup>
A.			
August	20	0 - 150.0	20.6
September	22	0 - 1,100.0	65.5
October	22	0 - 2,400.0 <sup>a</sup>	223.7
November	22	0 - 9.0	1.4
December	22	0 - 23.0	3.8
January	22	0 - 4.0	0.6
Mean for 6 Month Observations			53.0
B.			
August	20	0 - 28.0	7.3
September	22	0 - 1,100.0	193.2
October	22	0 - 2,400.0 <sup>a</sup>	289.6
November	22	0 - 23.0	6.9
December	22	0 - 93.0	12.7
January	22	0 - 150.0	15.2
Mean for 6 Month Observations			89.0

\* Escherichia Coli values expressed as M.P.N. of Escherichia coli /100 g.

<sup>a</sup> Quantity either equal or greater than listed.

A = As Purchased; B = After 7 Days Iced Storage;  
N = Number of Observations.



Table 4. Monthly Coagulase-Positive S. aureus Values\* For Oysters Purchased Over A Period Of Six Months

Month	N	Range	Mean
A.			
August	20	0 - 210	13.4
September	22	0 - 21	3.0
October	22	0 - 23	3.1
November	22	0 - 1,100	70.9
December	22	0 - 9	1.1
January	22	0 - 9	0.6
Mean for 6 Month Observations			15.5
B.			
August	20	0 - 240	50.9
September	22	0 - 15	4.3
October	22	0 - 23 <sup>a</sup>	5.4
November	22	0 - 2,400 <sup>a</sup>	219.4
December	22	0 - 21	4.3
January	22	0 - 75	6.0
Mean for 6 Month Observations			48.4

\* S. aureus Values expressed as M. P. N. of coagulase-positive S. aureus /g.

<sup>a</sup> Quantity either equal or greater than listed.

A = As Purchased; B = After 7 Days Iced Storage  
N = Number of Observations

A MICROBIOLOGICAL SURVEY OF  
COASTAL GEORGIA COMMERCIAL ICE PLANTS

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A microbiological survey of three major ice plants serving Georgia's shrimping fleet was conducted as a portion of the University of Georgia Marine Extension Service's on going program to assess and improve the sanitary and organoleptic quality of Georgia's seafood as it moves from harvest, through packing and processing, to the retail market place, and finally to the consumer. The survey was conducted between June, 1978 and March 1979 to ascertain the microbiological quality of commercial ice available for use by Georgia shrimpers in conjunction with a simultaneous study to develop simple and effective clean-up and sanitation practices aboard commercial shrimp boats.

Our involvement with ice plant sanitation began after Glynn County, Georgia adopted U.S. Public Health Service Ice Sanitation standards which required plants to meet Federal Safe Drinking Water standards. Plant operators and the Georgia Department of Agriculture requested assistance from The Marine Extension Service to evaluate plant operations in terms of the new regulations. Total coliform organisms detected during a prior limited sampling of ice aboard shrimp boats reinforced the need to evaluate the microbiological quality of ice produced along Georgia's coast. The ice plants were sampled at the water source, the final product, and at critical production points for total aerobic plate counts, total coliforms, fecal coliforms, and fecal streptococci populations to determine what changes, if any, were needed to improve product quality and to meet or exceed federal and state drinking water requirements (Georgia Department of Natural Resources Rules for Safe Drinking Water, Chapter 391-3-5, July, 1977). Water source and product samples were collected during the summer and winter at each plant to compare product quality during periods of high and low production, respectively.

Plant #1 is an ammonia-brine block ice plant with a capacity of seventy (70) tons of ice per day. Standard blocks of ice are produced from well water (with city water back-up) frozen in steel ice forms by immersion in ammonia cooled brine. The brine and block freezing area is covered by wooden sections that lift up for access to ice forms surrounded by brine. Production and storage are primarily manual operations conducted in two separate plant areas of similar design. Ice blocks are transported from the plant to docks serving fishing boats where they are crushed and blown aboard the boats. During the 1978 shrimping season the plant produced 62,000 blocks (approximately 9300 tons) of ice and served approximately two-hundred (200) fishing boats.

Plant #2 is of similar design to Plant #1, producing block ice from well water (with city water as an auxiliary source) in steel ice forms, frozen in ammonia cooled brine. The plant consists of a single unit with a daily capacity of seventy (70) tons. Block-ice produced by the predominantly manual operation is transported to docks serving the plant's clientele, where it is crushed and blown aboard fishing boats. Approximately seventy to eighty (70-80) boats were regularly supplied with ice during much of the 1978 shrimping season, however the operator of Plant #2 stated that competition from flake ice Plant #3, which opened on July 1, 1978, reduced the number of boats he supplied with ice by approximately eighty percent before the close of the shrimping season.

Plant #3 is a modern, automated flake ice facility with a daily capacity of sixty (60) tons. A Turbo ice-making machine charged with freon produces ice flakes on steel plates that are then broken loose from the plates and fall into an automated ice storage bin. A well with city water back-up provides water for ice production. The plant is located on the waterfront with a stainless steel and flexible plastic pipe connecting the plant to a dock where "snow ice", provided by a floor screw in the ice storage bin, can be pumped aboard waiting boats with a fifty (50) horsepower blower. The equivalent of approximately 60,000 blocks (9,000 tons) of ice were sold since the plant opened in July, and approximately fifty to sixty (50-60) boats were supplied on a regular basis during the shrimping season.

In addition to a survey of the microbiological quality of ice utilized by Georgia's shrimping fleet, the purpose of the study was to determine probable areas of contamination during ice production at each plant, and to bring about changes in employee practices and the

physical plant itself designed to improve the microbiological quality of the final product. Complete and anticipated tasks required to improve the microbiological quality of ice produced at each plant are outlined as follows:

- (a) Marine advisory agents apprised plant operators of manufacturing practices and physical plant construction that were determined to be sources of contamination following the microbiological examination of critical production points. The agents recommended any changes in the physical plant or production practices needed to improve product quality.
- (b) Training workshops for employees at each plant are being conducted by advisory agents to impress upon them the importance of personal and plant sanitation to the quality of ice produced.
- (c) Ice plants will be encouraged to institute needed improvements in production practices.
- (d) Product and critical production points will be sampled microbiologically after plant improvements are completed to determine their effectiveness.
- (e) Operators of each plant will be urged to participate in a regular quality control program.

#### MATERIALS AND METHODS

Duplicate 100 ml samples of water and/or ice were collected at various stages of production at each of the three plants for the determination of total coliform, fecal coliform, and fecal streptococci populations utilizing the membrane filter technique (1). Production surfaces were sampled with cotton swabs. A twenty-five (25) cm<sup>2</sup> sterile wire template was placed on a surface that was then sampled with a wet swab dipped in sterile phosphate dilution buffer (4) followed by swabbing with a dry cotton swab (2,3). Each swab sample consisted of twelve (12) strokes inside of the template in one direction, followed by twelve (12) strokes perpendicular to the first direction after rotation of the cotton swab 180°. Swabs were transported to the laboratory in sterile phosphate dilution buffer. Standard aerobic plate counts, MPN total coliforms, MPN fecal coliforms, and MPN fecal streptococci determinations were completed for each swab sample (1,4). Water, ice, and swab samples were transported to the laboratory on ice.

Samples were collected from the three plants on the following occasions:

- (a) Plant #1 was sampled five (5) times during summer and winter.
- (b) Plant #2 was sampled three (3) times during summer and winter.
- (c) Plant #3 was sampled three (3) times during summer and four (4) times during winter.

In addition to the three plants, ice samples were taken from shrimp boats during the summer on four (4) occasions after it was blown on board, but prior to contact with any fishery products. These samples were treated in the same manner as ice samples collected from the plants.

The range and the arithmetic mean were determined for all sample sets. Analysis of variance was utilized to determine any significant differences between plants, and at the same plant during winter and summer production. The following samples were examined statistically to complete the survey of ice quality: (i) water source, (ii) block ice (Plants #1 and #2), and flake ice (Plant #3), and (iii) crushed ice (7,8).

A sanitation workshop consisting of a lecture session and an audience participation session was conducted for employees of each ice plant by marine advisory personnel. The lecture session described good manufacturing practices and their relationship to product quality, explained the importance of bacteria and the effect of rapid microbial growth on product contamination, discussed the use and misuse of sanitizers, and emphasized the importance of personal hygiene. A series of slides were presented showing microbial growth on petri plates containing standard plate count agar and Levine EMB agar that were inoculated with dirty hands, clothing, gloves (inoculated with *E. coli*), money, boots, hair, and insects. A slide series on proper hand washing and sanitation was presented. Petri plates containing standard plate count agar were inoculated with dirty hands, hands after rinsing in tap water, hands after washing with soap and rinsing in tap water, and hands after washing with soap followed by a sanitizer ("Tamed Iodine" Scrub, West Chemical Products, Inc., New York, N.Y.)\* and a tap water rinse. The plates were incubated at 35 C for forty-eight (48) hours before photographing. The slides show the

\*The use of any product name does not imply endorsement by the University of Georgia Marine Extension Service.

large number of organisms that remain on the hands after rinsing in tap water or washing with soap. The effectiveness of hand sanitizers was impressed upon the employees. A similar series of slides was presented showing Levine EMB plates inoculated with gloves contaminated with E. coli.

During the participation portion of the workshop, advisory agents provided individual workers with petri plates containing standard plate count agar. The employees inoculated the plates with their hands, portions of their clothing, money, and with their hair. The plates were collected by the advisory agents who returned forty-eight (48) hours later after incubating the plates at 35 C. Microbial growth on the plates provided a graphic demonstration to each worker concerning his ability to contaminate the final product when proper sanitation procedures are not followed.

## RESULTS AND DISCUSSION

- I. Georgia Department of Natural Resources Rules for Safe Drinking Water, Chapter 391-3-5, July, 1977. Section 391-3-5-.18. Primary Maximum Contaminant Levels for Drinking Water. Amended (5), sets the following maximum limits on coliform populations to be found in drinking water which applies to commercial ice as well:
  - 4) Maximum microbiological contaminant levels for coliform bacteria, applicable to community water systems and non-community water systems are as follows:
    1. When the membrane filter technique pursuant to Section 391-3-5-.19(1) is used, the number of coliform bacteria shall not exceed any of the following:
      - (i) One per 100 milliliters as the arithmetic mean of all samples examined per month pursuant to Section 391-3-5-.19(2)
      - (ii) Four per 100 milliliters in more than one sample when less than 20 are examined per month; or
      - (iii) Four per 100 milliliters in more than five percent of the samples when 20 or more are examined per month.

## II. Microbiological Survey of Ice Quality

The microbiological data collected at block ice Plants #1 and #2 for water source, block ice, and crushed ice samples during the summer and winter sampling periods was combined in Table 1. Plants #1 and #2 are of similar wooden construction and follow the same production pattern to produce standard blocks of ice using vintage 1930 ammonia-brine compressor systems. The arithmetic mean and range of total coliform, fecal coliform, and fecal streptococci organisms recovered from the water sources, block ice, and crushed ice from Plants #1 and #2 sampled during summer's peak production and winter's minimal production period are presented in Table 1. During the summer sampling period the block ice Plants (#1 and #2) exceeded state total coliform standards for water source, block ice, and crushed ice (Table 1) with arithmetic mean total coliform values of 10.0 organisms/100 ml, 4.0 organisms/100 ml, and 36.0 organisms/100 ml, respectively. The arithmetic mean of total coliform populations detected during the winter for the water source (0.6 organisms/100 ml), block ice (0.7 organisms/100 ml), and crushed ice (0.5 organisms/100 ml) were within state guidelines, however the maximum permissible value of four (4) coliforms per 100 ml was exceeded for the combined water source (Table 1).

Automated flake ice production using a freon system combined with the recent construction of Plant #3 led to a natural separation of product and critical quality control point microbiological data collected at Plant #3 from data obtained at block ice Plants #1 and #2. The arithmetic mean and range of total coliform, fecal coliform, and fecal streptococci organisms recovered from the water source, flake ice storage bin, and crushed ice from Plant #3 during summer and winter at similar levels of production are presented in Table 2. Water source coliform values for Plant #3 were acceptable during summer and winter sampling along with coliform values from the flake ice storage bin and crushed ice samples obtained during the winter. Summer mean coliform values of 3.7 organisms/100 ml for the ice storage bin and 5.3 organisms/100 ml for crushed ice exceeded state standards. A maximum of 12 coliform organisms/100 ml was obtained for flake ice and crushed ice summer samples (Table 2).

<u>Location</u>	<u>Total Coliforms</u> <u>100 ml</u>		<u>Fecal Coliforms</u> <u>100 ml</u>		<u>Fecal Strep</u> <u>100 ml</u>	
	<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range</u>
<u>Water Source</u>						
Summer	10.0 <sup>b</sup>	0-54	0.3	0-2	103.5 <sup>b</sup>	0-580
Winter	0.6 <sup>b</sup>	0-9	0.3	0-3	6.3 <sup>b</sup>	0-30
<u>Block Ice</u>						
Summer	3.9	0-30	0.1	0-2	167.5	2-883
Winter	0.7	0-2	0.1	0-1	130.7	7-900
<u>Crushed Ice</u>						
Summer	36.0	1-117	0.5	0-2	767.1 <sup>a</sup>	61-1490
Winter	0.5	0-2	0.0	0	74.7 <sup>a</sup>	6-147

<sup>a</sup>Summer vs winter significant at the 0.01 level.

<sup>b</sup>Summer vs winter significant at the 0.05 level.

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Table 1. BLOCK ICE PLANTS #1 and #2, Combined  
Microbiological Survey of Ice Quality.



<u>Location</u>	<u>Total Coliforms</u> 100 ml		<u>Fecal Coliforms</u> 100 ml		<u>Fecal Strep</u> 100 ml	
	<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range</u>
<u>Water Source</u>						
Summer	0.0	0	0.0	0	0.0	0
Winter	0.0	0	0.0	0	9.8	0-31
<u>Ice Storage Bin</u>						
Summer	3.7	0-12	0.0	0	6.7	0-16
Winter	0.0	0	0.0	0	0.3	0-1
<u>Crushed Ice</u>						
Summer	5.3 <sup>b</sup>	0-12	0.0	0	35.3 <sup>b</sup>	4-66
Winter	0.0 <sup>b</sup>	0	0.0	0	3.0 <sup>b</sup>	0-8

<sup>b</sup>Summer vs winter significant at the 0.05 level

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Table 2. FLAKE ICE PLANT #3, Microbiological Survey of Ice Quality.

The data from the ice quality survey indicated that all three ice plants exceeded state and federal maximum permissible total coliform levels for stored block ice (Plants #1 and #2) or stored flake ice (Plant #3) and crushed ice during the summer at peak production before the plant operators were advised of any production problems and before any sanitation workshops were completed. Significant differences between coliform and fecal streptococci populations during summer and winter and from plant to plant indicated that improvements could be made in the microbiological quality of ice produced for Georgia's shrimp industry. A limited survey of ice on board shrimp boats revealed coliform levels in excess of state and federal standards. In addition to a microbiological survey of the ice plants, it was decided that an educational program would be developed by Marine Extension personnel and plant operators to improve ice quality.

### III. Plant Problems, Clean-up, and Improvements

Ice production facilities, manufacturing practices, and employee habits were observed at the three cooperating ice plants. Critical production points were determined for each plant. The microbiological loads at these points and the observations and suggestions from marine advisory agents were combined to delineate specific problems in each plant and to offer specific improvements designed to reduce ice quality problems.

The following recommendations designed to improve the quality of block ice produced at block ice Plants #1 and #2 were made to the operators:

- (a) Institute chlorination of well water used to supply the plant or switch to city water.
- (b) Clean-out, sanitize, and cover the ice form filling tank.
- (c) Scrub out ice filling forms with soap and sanitize by dipping into a 200 ppm chlorine solution before refilling (6).
- (d) Paint the wooden covers of the block freezing area with non-leaded enamel paint.
- (e) Wash and sanitize the covers on a regular basis and reduce worker traffic through the area to those employees actively concerned with ice production.

- (f) Insist that the worker lowering the ice forms into the thawing vat wear clean rubber boots and that he step through a 200 ppm solution of chlorine before his feet come in contact with the ice form(6).
- (g) Line the wooden thawing vat with fiberglass or other suitable material. Wash and sanitize the vat on a regular basis.
- (h) Detergent wash and sanitize ice storage freezer floor on a regular basis.
- (i) Replace dripping and rusting pipes in the storage area.
- (j) Install a sanitary ceiling over the block freezing area.
- (k) Although the ice crusher appeared to be microbially clean, a regular regime of washing and sanitation should be instituted.

The operator of Plant #1 expressed an interest in implementing needed physical improvements and changes in plant production. He noted an improvement in worker attitude and performance after completion of the first employee sanitation workshop at his plant.

During the initial summer survey, marine advisory agents discovered a leak in the stainless steel pipe running from the blower to the dock at flake ice Plant #3. "Snow ice" blown aboard shrimp boats was contaminated with soil pulled into the pipe. Mean values of 17.0 total coliforms/100 ml, 1.6 fecal coliforms/100 ml, and 231.0 fecal streptococci/100 ml were determined for the boat ice (Table 3). The operator of Plant #3 (i) repaired the leak in the ice transport pipe, (ii) cleaned and sanitized the Turbo ice maker, and (iii) cleaned and sanitized the ice storage bin before we began winter sampling. No coliforms or fecal coliforms were detected in the water source, flake ice storage bin, crushed ice, or ice blown aboard boats (Tables 2 and 3) at Plant #3 during the winter sampling period. Mean fecal streptococci levels were reduced to 0.3 organisms/100 ml in the ice storage bin, 3.0 organisms/100 ml in the crushed ice, and 11.5 organisms/100 ml in ice blown on board the boats. No additional changes were recommended.

# ICE SAMPLES

<u>Location</u>	<u>Total Coliforms</u> <u>100 ml</u>		<u>Fecal Coliforms</u> <u>100 ml</u>		<u>Fecal Strep</u> <u>100 ml</u>	
	<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range</u>
Ice Blown On Boat Before Clean-up	17.0	4-26	1.6	0-6	231.0	206-274
Ice Blown On Boat After Clean-up	0.0	0	0.0	0	11.5	4-19

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Table 3. FLAKE ICE PLANT #3, Microbiological Loads  
at Critical Quality Control Points.

## CONCLUSIONS

Ice produced by three major coastal ice plants, serving two-thirds (2/3) of Georgia's shrimp boats during the 1978 season exceeded state and federal microbiological standards. In addition to potential problems that could develop between the ice producing industry in coastal Georgia and state and local regulatory agencies which include (i) regulatory actions and (ii) cost of compliance with present regulations, poor ice quality poses a problem for the remainder of the seafood industry. Microbial contamination of seafood products during initial storage on-board fishing vessels certainly contributes to deterioration of product quality and shortening of shelf life.

The operators of the three ice plants examined during the study expressed an interest in improving the quality of ice produced at their plants. The Marine Extension Service, through its advisory agents, offered technical assistance and provided specific plans for production and physical improvements to each operator on a voluntary basis. Flake ice plant #3, the most modern facility, implemented clean-up and sanitation procedures and a simple modification of its ice blowing system to comply with state and federal microbiological regulations. Block ice plants #1 and #2 (of an older labor intensive design) will require more extensive modifications in physical construction and continued training of employees in good manufacturing and sanitation practices to achieve a better quality product.

## ACKNOWLEDGEMENTS

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# "DIP STICK" METHOD FOR MONITORING FECAL COLIFORM LEVELS IN SHELLFISH <sup>1/</sup>

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Analyses of shellfish for fecal coliforms involves the most probable number (MPN) technique (1-3) which is relatively cumbersome, requiring numerous tubes of culture media and multiple transfers. The seafood industry is often unable to use this technique because of its complexity and the high cost of labor. In addition, the time required to obtain MPN results can be as long as 72 h.

A newly developed "dip stick" technique has recently been published by Richards (6) on the use of Millipore Coli-Count Samplers <sup>2/</sup> (Millipore Corp., Bedford, Mass.) for monitoring fecal coliform levels in oysters. Another paper by Richards (in press) deals with the use of Coli-Count Samplers for enumeration of fecal coliforms in blue crab meat.

This report explains the Coli-Count Sampler technique and summarizes the advantages and disadvantages of the technique as compared to MPN procedures for the enumeration of fecal coliforms in oysters, shrimp, and blue crab meats.

Samplers consist of a plastic tab which supports a 0.45  $\mu$ m membrane filter bonded to an underlying absorbent pad (Fig.1). The pad contains dehydrated culture media. For inoculation the tab is immersed in the solutions to be tested for 30 sec to 5 min depending on the amount of filter clogging materials in the liquid (5). Then 1.0 ml of liquid passes through the filter and into the pad. During this process bacteria are trapped on the

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<sup>1/</sup> Contribution no. 79-27C. National Marine Fisheries Service, Southeast Fisheries Center, Charleston, S.C. 29412.

<sup>2/</sup> Use of trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

filter's surface (Fig. 2). Nutrients in the pad then diffuse up to the surface of the filter and provide the essential nutrients to promote coliform growth (Fig. 3). The sampler is then placed in an incubator at  $44.5 \pm 0.2^{\circ}\text{C}$  for 18-24 h (4).

#### MATERIALS AND METHODS

Fresh Eastern oysters, Crassostrea virginica; fresh white shrimp, Penaeus setiferus; and commercially processed meat of blue crabs, Callinectes sapidus, were obtained from local retail outlets.

Fecal coliform counts were negligible in most preliminary samples tested. Samples were, therefore, inoculated with fecal coliforms according to the following procedures. Live oysters were placed in an artificial seawater system containing fecal coliforms and allowed to ingest the inoculated water for 24-48 h prior to analyses (6). Shrimp and blue crab meats were inoculated by adding aliquots of coliform cultures directly into the blender jars prior to blending. All homogenates were 1:10 dilutions prepared with 25 g of shellfish meat and 225 ml of sterile peptone buffer (0.1%, Difco) and blended for 2 min at high speed. Serial dilutions were prepared with 90 ml peptone buffer dilution blanks. Three tube MPN's were performed according to the procedures in the Compendium of Methods for the Microbiological Examination of Foods (2) using Difco or Baltimore Biological Laboratories' media.

Coli-Count Sampler's tabs were weighed prior to inoculation. Then duplicate samplers were immersed for 5 min in the shellfish homogenate and in a 10-fold dilution of the homogenate (1:100) for each sample tested. After inoculation tabs were shaken three times to remove excess liquid and reweighed to determine the approximate volumes of sample absorbed. Samplers were incubated according to manufacturer's instructions (4), after which blue or blue-green colonies (4,5) and yellow-green colonies were counted as fecal coliforms.

MPN tubes and Coli-Count Samplers were inoculated with the upper portion of the crab homogenate because crab particles settling to the bottom of the blender jar could block the sampler's filter membrane and prevent absorption of the required 1.0 ml of sample. Due to excessive foaming, shrimp homogenates were allowed to settle 1.5 min after blending to permit a liquid layer to form and this liquid layer was used as inoculum. Oyster homogenates were randomly sampled throughout the liquid portion.

#### RESULTS

Based on the required absorption of 1.0 ml of liquid per sampler, Table 1 shows that the absorption of oyster homogenates by samplers was significantly impaired due to blockage of the samplers' filter membranes with oyster particles. Absorption



of crab and shrimp homogenates was not impaired. Significant impairment of absorption also was not observed among the 1:100 dilutions tested.

All fecal coliform counts derived from Coli-Count Samplers fell within the corresponding MPN's 95% confidence interval with only one exception. Counts obtained from oyster homogenates were, in some cases, lower than the corresponding MPN's 95% confidence limit. This is attributable to insufficient absorption of oyster homogenates by the samplers due to blockage of the filter membranes.

MPN and sampler counts were standardized by a  $\log_e$  transformation (7). A significant correlation was observed for fecal coliform counts between MPN's and Coli-Count Samplers for all shellfish samples except oyster homogenates. The correlation coefficients of the transformed data were 0.883 for blue crab, 0.959 for shrimp, and 0.966 for 1:100 and higher dilutions of oyster homogenates.

## DISCUSSION

The use of Coli-Count Samplers for monitoring fecal coliform levels in oyster, shrimp and blue crab homogenates and/or dilutions of homogenates has advantages and disadvantages compared to MPN testing procedures.

### Advantages of Samplers

Samplers are faster than MPN's requiring only 18-24 h per analysis. Transfers are not required thus decreasing the chance of error and also decreasing labor involved in performing each test. Samplers do not require multiple culture tubes which are cumbersome and which may be misinterpreted as positive due to dissolved gasses from the media accumulating in the fermentation tubes during incubation. Samplers require only one reading after 18-24 h whereas MPN's require readings of gas positive tubes after 24, 48, and 72 h. These and other savings in labor make the sampler a more cost effective alternative to the MPN procedure.

Statistical tables are not required. Samplers enumerate the actual viable coliforms rather than determine a statistical range or probable count of coliforms as with MPN's.

Samplers are more applicable for field work than MPN's. They are small enough to fit several in a pocket. A portable incubator specifically designed for the sampler is available and may prove useful for field work.

The sampler provides industry with an alternate method for coliform analyses and may provide small processors the ability to test their own products. Personnel with no technical training should find the theory and operation of the sampler less com-

plex than that of the MPN's.

#### Disadvantages of Samplers

Samplers cannot be used to enumerate fecal coliforms less than 10/g for shrimp and blue crab meats or less than 100/g for oyster samples because of the shellfish dilutions tested.

The color of the coliform colonies on the samplers may differ from the blue or blue-green color specified by the manufacturer. When coliform counts are very high, there appears to be an insufficient amount of indicator dye in the sampler to properly stain the colonies. This results in a green or yellow-green color. In addition, if heavy concentrations of seafood particles are present on the surface of the sampler, then particles will absorb sufficient dye to result in green or yellow-green colony coloration.

Representative blue, blue-green, green, yellow-green and yellow colonies on samplers were subjected to biochemical tests for confirmation as fecal coliforms. All colonies were verified as fecal coliforms except the yellow colonies on oyster, shrimp, and blue crab inoculated samplers which were not coliforms. Therefore, the color disparity of colonies on samplers is not a serious disadvantage providing yellow colonies are not counted as fecal coliforms.

#### CONCLUSION

Coli-Count Samplers can be used for enumerating fecal coliforms in 1:100 oyster homogenate dilutions. They can also be used for 1:10 crab homogenates when the samples are taken from the upper portion of the mixture, and for 1:10 shrimp homogenates when the samples are taken from the liquid portion of the mixture which forms approximately 1.5 min after blending. Higher dilutions of oyster, shrimp, and blue crab homogenates can be readily analyzed for fecal coliforms without concern for filter membrane blockage.

Routine use of samplers should be preceded by tests to access the effects of shellfish particles on the absorption of appropriate volumes of sample since freezing, length and temperature of storage, differences in processing methods, geographic distribution, and seasonal variations may affect textural and other physical characteristics of shellfish and hence absorption of the homogenate by the samplers.

Suggested applications of the sampler include (i) use at critical control points throughout a processing operation, (ii) determining the range of coliforms prior to performing MPN's, (iii) assaying for fecal coliform contamination above regulatory guidelines, and (iv) indirectly monitoring sanitary aspects of transport, holding, storage, and processing facilities.

Processors and handlers of shellfish, State and Federal health agencies, and field inspectors may find the Coli-Count Sampler a useful and practical testing method to achieve their analytical objectives.

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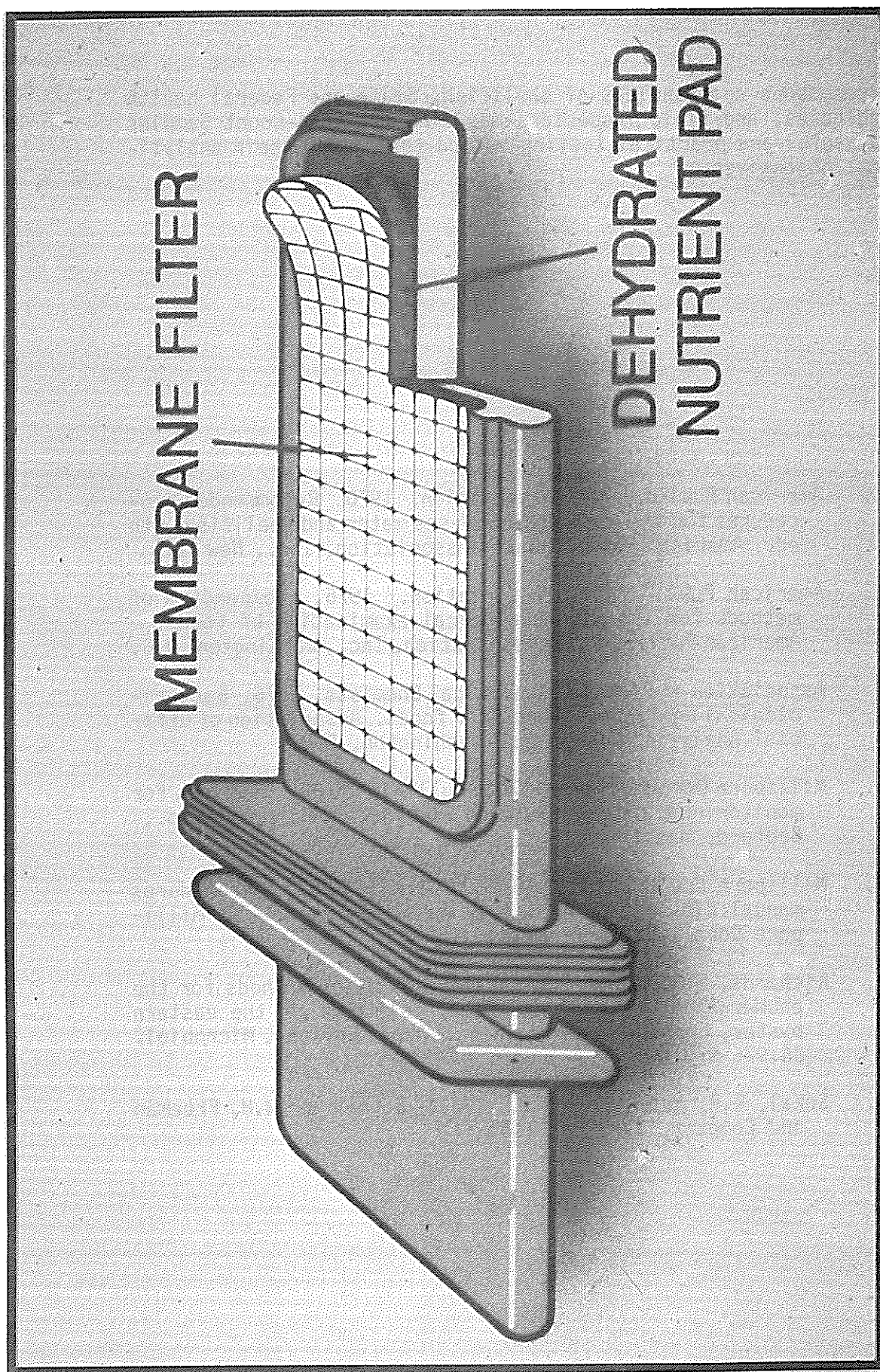


Figure 1. Crosscut view of Coli-Count Sampler.  
(Reprinted by permission of Millipore Corp.)

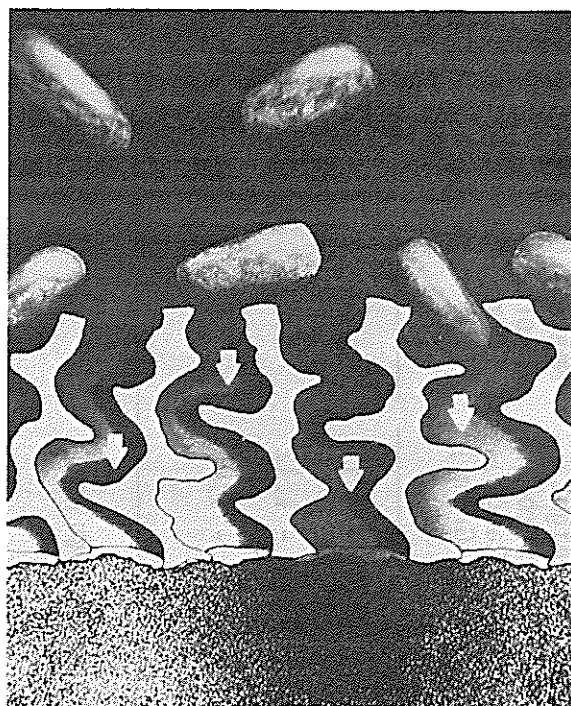


Figure 2. Crosscut view of Coli-Count Sampler absorbing liquid and trapping bacteria in upper portion of filter membrane. (Reprinted by permission of Millipore Corp.)

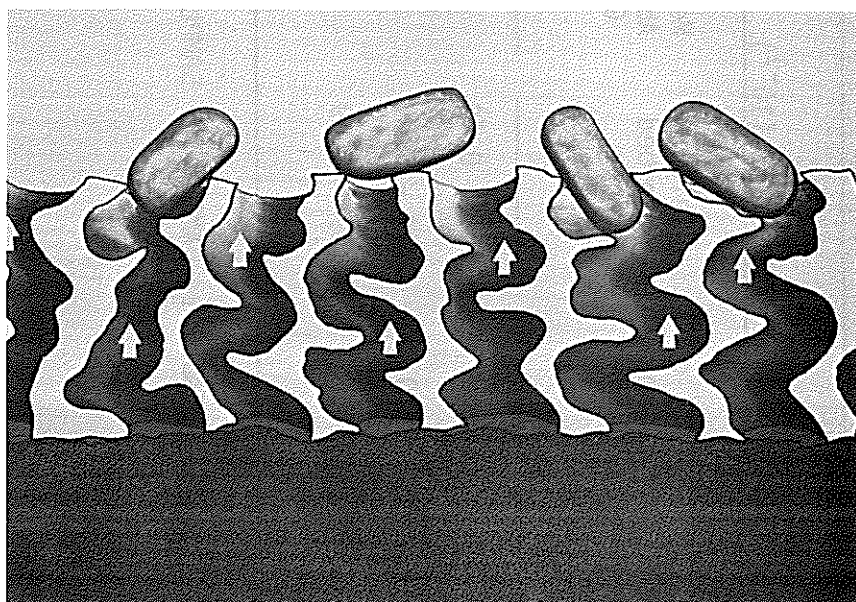


Figure 3. Coliform growth media in absorbent pad diffusing through Coli-Count Sampler's filter membrane to the surface bacteria. (Reprinted by permission of Millipore Corp.)

SAMPLES	HOMOGENATE ( $10^{-1}$ )			DILUTION ( $10^{-2}$ )		
	No. of Samples Tested	Weight Absorbed (Range in Grams)	Mean Wt. Absorbed Grams	No. of Samples Tested	Weight Absorbed (Range in Grams)	Mean Wt. Absorbed Grams
OYSTER	10	0.39 - 0.53	0.47	10	1.02 - 1.11	1.05
BLUE CRAB	30	0.94 - 1.09	0.99	30	0.97 - 1.12	1.03
SHRIMP	10	0.99 - 1.09	1.04	10	0.97 - 1.08	1.04

TABLE I. AMOUNT OF SAMPLE ABSORBED BY COLI-COUNT SAMPLERS.

## PLANOCOCCUS CITREUS: ITS POTENTIAL FOR SHRIMP SPOILAGE

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The taxonomic status of Planococcus citreus has changed markedly in the past fifteen years. In 1894 and again in 1900, Migula (27,28) made a recommendation that flagellated cocci be included either in the genus Planococcus or Planosarcina. This concept was accepted by only a few authors, e.g., Krasil'ni kov in 1949 (22). The majority of authors have included the flagellated cocci in the genus Micrococcus (18,24) mainly, because these cocci could only be differentiated from the other members of the genus by their motility. Most authors have considered motility to be too minor of a characteristic for the recognition of a new genus. The findings of Bohacek et al. (6,7) that the flagellated cocci differ considerably in the GC content of their DNA from other cocci threw new light on their taxonomic position. It was therefore proposed by Bohacek et al. (6) to include the flagellated cocci with a GC content ranging from 40-50% in the genus Planococcus. In 1970, Kocur et al. (21), revised and clearly outlined the genus Planococcus.

The Eighth edition of Bergey's Manual of Determinative Bacteriology (8) states that Planococcus citreus is a motile, gram positive coccus of marine origin. There appears to be very little information in the literature describing the presence of this organism in the marine environment as well as information on the isolation and characterization of this bacterium. The only report that has come to our attention that relates to the isolation of P. citreus is that of Cook (14). He mentioned the isolation of motile pigmented cocci from shrimp. Like previous workers (18,19), he placed these organisms in the genus Micrococcus. Our attention was directed toward this organism, when during a study of the normal flora of Sicyonia brevirostris, P. citreus was isolated consistently and found to increase in numbers during iced storage (26). In this study, 68% of the isolates recovered were gram positive cocci, with P. citreus increasing from 10% of the isolates on the fresh shrimp to 40% on the stored shrimp.

In that isolation of gram positive organisms from iced seafood is unusual, this report outlines observations on the distribution of P. citreus in the marine environment as well as some growth and isolation characteristics of interest to the fisheries microbiologist.



## MATERIALS AND METHODS

### Isolation and characterization of *P. citreus*

Following numerous unsuccessful attempts at developing a selective medium for *P. citreus*, Plate Count agar with 0.5% sodium chloride and 0.5% gelatin was selected as the primary isolation medium. Large (150 mm) petri dishes were poured, dried overnight and diluted samples spread over the agar surface. Incubation was at 25°C for 48 hours. Small yellowish-orange colonies surrounded by a visible zone of gelation hydrolysis were picked to Plate Count agar slants. All media contained at least 0.5% sodium chloride. Isolates were Gram stained and checked for glucose fermentation in OF (19) and MOF (23) media. Growth was determined at various salt (0-18%) and pH (6-10.9) levels in Plate Count broth. All isolates were checked for flagella as outlined by Difco (15). Protein hydrolysis studies were conducted in Nutrient agar with 0.5% added protein. Confirmation of hydrolysis was made following flooding of the plates with acidified mercuric chloride.

Irradiation: A number of preliminary studies were conducted to determine the appropriate dose for the maximum elimination of microorganisms from the shrimp. It was finally concluded that approximately 600 Krad was adequate. Before irradiating, the shrimp were placed in sterile Whirl-Pack<sup>(R)</sup> Bags. A Mark III 'Preco' Lodi Irradiator was designed by Brookhaven National Laboratories and built by Processing Equipment Corporation, Lodi, N.J., was used. The bags with the shrimp were placed in the middle of three stainless steel containers. After sealing of the containers, they were lowered approximately 14 ft under water and subjected to gamma rays for 4 hr at 20°C.

Culture: A culture of *Planococcus citreus* "A-17" was selected for this study. The culture for inoculation of the irradiated shrimp was grown on Plate Count agar slants at 20°C for 24 hr. Dilutions were made in sterile water to give a final concentration of approximately  $10^3$  organisms per gram/shrimp following dipping.

Microbiological Analyses: Aerobic plate counts were conducted after 0, 4, 8, 12, 16 days of storage at 5°C. Plate Count agar with 0.5% NaCl added was used with incubation at 20°C for 5 days. Methods outlined in the Compendium of Methods for the Microbiological Examination of Foods (2) were followed. All analyses were done in triplicate.

Biochemical Analyses: Selected biochemical tests were performed in order to assess the contribution of this organism to the spoilage of *Penaeus* shrimp. Tests were conducted after 0, 4, 8, 12, and 16 days of storage at 5°C.

a) Shrimp extracts: Shrimp extracts were prepared in a Waring Blendor from at least five shrimp at a ratio of 1 g of shrimp to 2 ml 7% TCA. The homogenate was then filtered through a Whatman #4 filter and the filtrate preserved at 5°C until used. For amino acid analyses, the supernatant was centrifuged after 5°C storage to remove additional protein.



b) Microdiffusion analyses of volatile nitrogen: The modified Conway (13) microdiffusion dish (30) was utilized for volatile nitrogen analyses. All analyses were conducted in triplicate. Values were multiplied by 1.3 to correct for incomplete distillation (11,12). Results are expressed as Mg volatile nitrogen/100 g of shrimp.

c) Amino acid nitrogen: Amino nitrogen was analyzed by a modification of the copper procedure of Spies and Chambers (32). Modifications were those outlined by Cobb et al. (11). Solutions were made following specifications set by Pope and Stevens (31). A standard curve was made following Cobb et al. (11). Results are expressed as millimoles amino nitrogen/100 g shrimp.

## RESULTS AND DISCUSSION

Of the 35 samples of marine origin examined for Planococcus citreus, only 5 yielded this organism. Four were shrimp samples and the fifth was a stuffed flounder sample that had been prepared in a plant that predominately processed shrimp. One of the shrimp samples from which Planococcus was isolated had been in frozen storage for at least six years. Fresh seafood (trout, sheepshead, mackerel, crab and oysters) as well as Gulf Coast waters and sediments from the vicinity of Suwannee, Florida, were also examined without success. However, in more recent studies performed by Mallory et al. (26), Planococcus citreus was isolated from estuarine areas in low numbers.

Extensive studies on the microbial flora of shrimp (3,4,5) have clearly shown that Planococcus citreus is an important part of the natural flora of shrimp. In these studies, shrimp were obtained from both coasts of Florida, from two different processors with different methods of handling shrimp. Twenty-two different bacterial genera and thirty-eight different species were found on the shrimp. Planococcus accounted for 13-24% of the isolates identified, depending upon how the shrimp were handled.

The recognition of P. citreus during the study of the normal flora of S. brevirostris and Penaeus shrimp, and the ability of this organism to grow on shrimp during iced storage raised the question as to whether this organism could contribute to the spoilage of this important marine food product. Protein utilization studies (Table 1) showed that this organism is capable of hydrolyzing cottonseed, soy and shrimp protein in addition to gelatin. Some of the isolates were also able to degrade whey and hog-blood isolate after extended incubation. Casein, barley, fish, peanut and corn-germ protein were unaltered. These data would indicate that Planococcus is capable of contributing to the alteration of shrimp protein during storage.

In addition, P. citreus is able to produce visible growth in Trypticase Soy broth at 5°C within 48 hr, supporting the observation of its psychrotrophic tendency (20). Planococcus will grow at 35°C but not 40°C with the best growth occurring between 25 and 30°C.

One of the most interesting characteristics of P. citreus is the pH range over which growth will occur. The organism will grow on Plate Count agar at a pH of 6.5 but not in broth at this pH. Growth occurred with all isolates at a pH of 10.1 but not 10.9. Shrimp protein is usually alkaline, about pH 7.0-7.5, and, therefore, within the range of active growth. Some isolates grow slowly in 0% salt medium with most requiring at least 0.5% salt and tolerating 12% salt without difficulty.

Recognition of Planococcus is difficult in that flagellation must be demonstrated. In media commonly used to demonstrate motility, the organism generally appears non-motile (Table 2) and interpretation of results is difficult. This may be due either to the aerobic nature of P. citreus or to its weak motile tendencies. The organism produces small yellowish-orange colonies on most media, which upon extended incubation increase to 3 mm or more in diameter. The organism is gram positive, appearing as single cells, pairs and tetrads. The cells are 1.0 to 1.5  $\mu$  in diameter. A limited series of biochemical tests are used to identify the organism. Tubes of glucose OF and MOF media and tubes of Plate Count broth adjusted to pH 6.5 and 7.5 are inoculated. Planococcus is negative for acid production in OF, produces acid oxidatively in MOF and does not grow in Plate Count broth at a pH of 6.5. After staining, one or two delicate flagella per cell can be observed. This organism can easily be misidentified if motility and flagellation are not determined.

Quality changes that occur in shrimp during storage are generally considered to result from the combined action of tissue enzymes and microbial contamination (16,29,33,25,9,17). Cobb and Vanderzant (9) studied selected biochemical changes that took place in alcohol sterilized shrimp inoculated with strains of Pseudomonas, Bacillus and coryneform bacteria. In our study we utilized gamma rays to lower bacterial numbers in shrimp before inoculation with Planococcus citreus.

Changes in the aerobic plate counts of control (natural flora), irradiated control and the irradiated/inoculated shrimp during 16 days of storage at 5°C are shown in Figure 1. P. citreus counts increased in the inoculated shrimp from  $5.0 \times 10^3$  bacteria/gram at 0 day to  $1.9 \times 10^8$  bacteria/gram at the 16th day. Aerobic plate counts of control samples increased rapidly. By the 16th day of storage, the control shrimp had  $2 \times 10^9$  bacteria/gram. Serious odor defects appeared by the 8th day of storage in control samples, while the Planococcus inoculated shrimp showed slight odor defects only by the 12th day of storage. The irradiated control sample had acceptable odor at the end of the storage period.

It is interesting to note that the irradiated control contained 200 bacteria/gram after irradiation. The National Research Council's Committee on Microbiology of Foods (1) has discussed the radiation resistance of a variety of organisms, i.e., Acinetobacter calcoaceticus, Micrococcus radiodurans, Micrococcus radiophilus, Moraxella osloensis, and Streptococcus faecium. Thornley (34) added some members of the Achromobacter-Alcaligenes group and some Bacillus species. A number of these bacteria are part of the normal flora of Penaeus shrimp.

Cobb and Vanderzant in 1975 (10) showed that the TVN/AAN ratio and logarithm of bacterial counts increased at approximately the same rate after the initial lag phase of bacterial growth. They suggested that a TVN/AAN ratio greater than 1.3 indicated a short shelf life for shrimp (12). Figure 2 shows the changes in TVN/AAN ratio in shrimp stored at 5°C for 16 days. By the 10th day of storage, the TVN/AAN ratio of the shrimp inoculated with P. citreus was above 1.3, similar in rate to the control shrimp. The irradiated control shrimp had a TVN/AAN ratio of 1.05 at the 16th day. Thus, if the TVN/AAN ratio is an indicator of shrimp quality, Planococcus citreus is capable of affecting the quality of Penaeus shrimp.

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# Protein Source

Isolate	Gelation	Whey	Cottonseed	Casein	Shrimp	Fish	Peanut	Corn germ	Soy	Hog blood isolate	Barley
A 17	<sup>1</sup> +	+	+	-	+	-	-	-	+	+	-
E 4	+	+	+	-	+	-	-	-	+	-	-
E 1	+	+	+	-	+	-	-	-	+	+	-
E 7	+	+	+	-	+	-	-	-	+	+	-
F 9	+	-	+	-	+	-	-	-	+	+	-
F 15	+	-	+	-	+	-	-	-	+	-	-
F 18	+	-	+	-	+	-	-	-	+	-	-
KS-1	+	-	+	-	+	-	-	-	+	-	-
KS-2	+	+	+	-	+	-	-	-	+	-	-
KS-3	+	-	+	-	+	-	-	-	+	-	-
KS-4	+	+	+	-	+	-	-	-	+	-	-
CS-1	+	-	+	-	+	-	-	-	+	+	-

<sup>1</sup>+ = hydrolysis; - = no hydrolysis

Table 1: Hydrolysis of various protein sources by Planococcus citreus at 25°C.

Medium	Culture									
	1	2	3	4	5	6	7	8	9	10
SIM <sup>1</sup>	- <sup>3</sup>	-	-	-	-	-	-	-	-	-
Motility test <sup>1</sup>	-	-	-	-	-	-	-	-	-	-
Motility S <sup>1</sup>	NG	NG	NG	NG	NG	NG	+	+	+	+
Motility nitrate <sup>2</sup>	-	-	+	-	-	-	-	-	-	-
Motility test <sup>2</sup>	+	-	+	-	-	+	-	+	-	+

<sup>1</sup>Difco            <sup>2</sup>Compendium (1)

<sup>3</sup>- = not motile

± = possible motile

NG = no growth

Table 2: Demonstration of motility by Planococcus citreus in various media following 7 days incubation at 25°C.



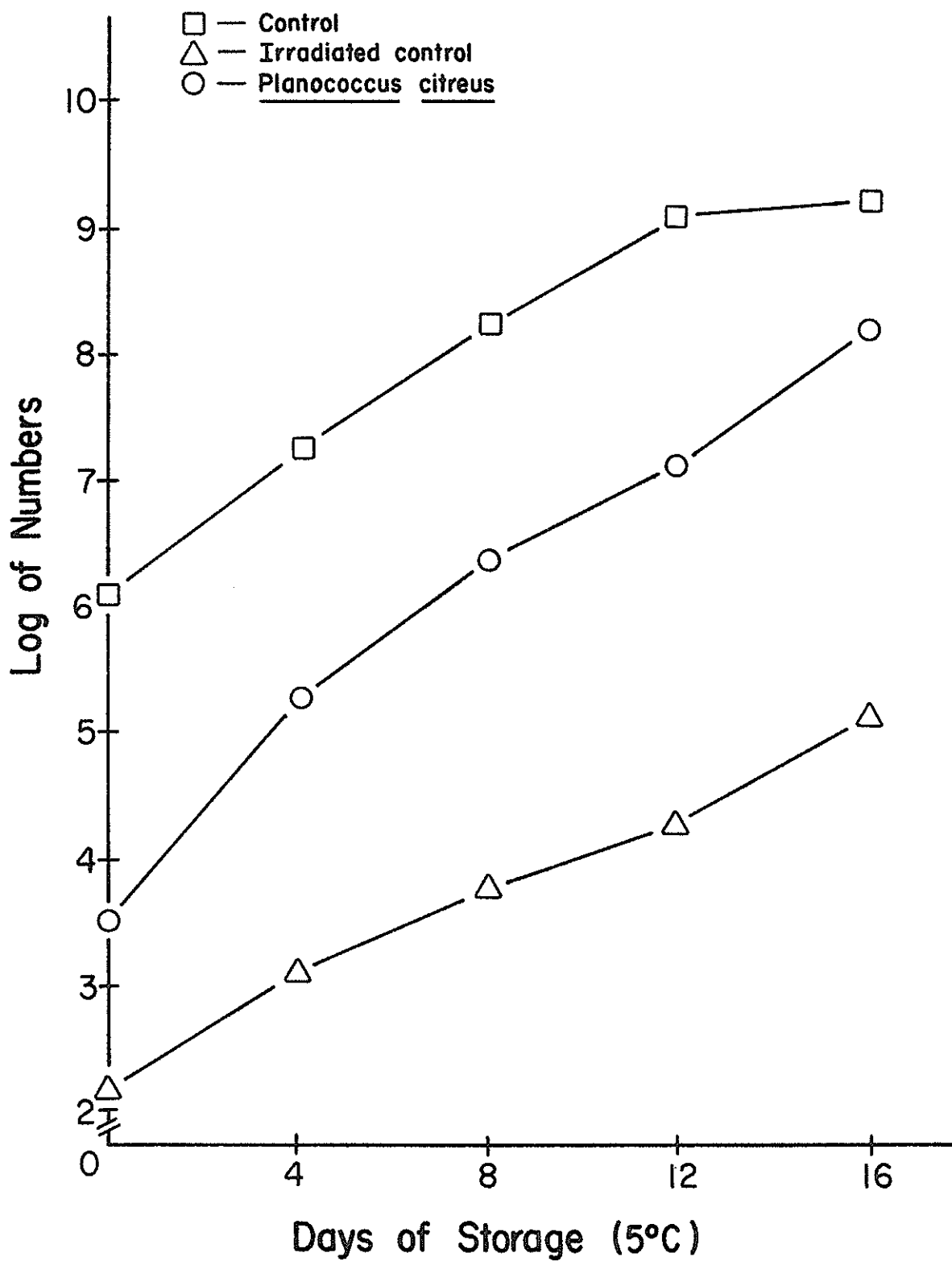


FIGURE 1. AEROBIC PLATE COUNTS OF SHRIMP STORED AT 5°C FOR 16 DAYS.

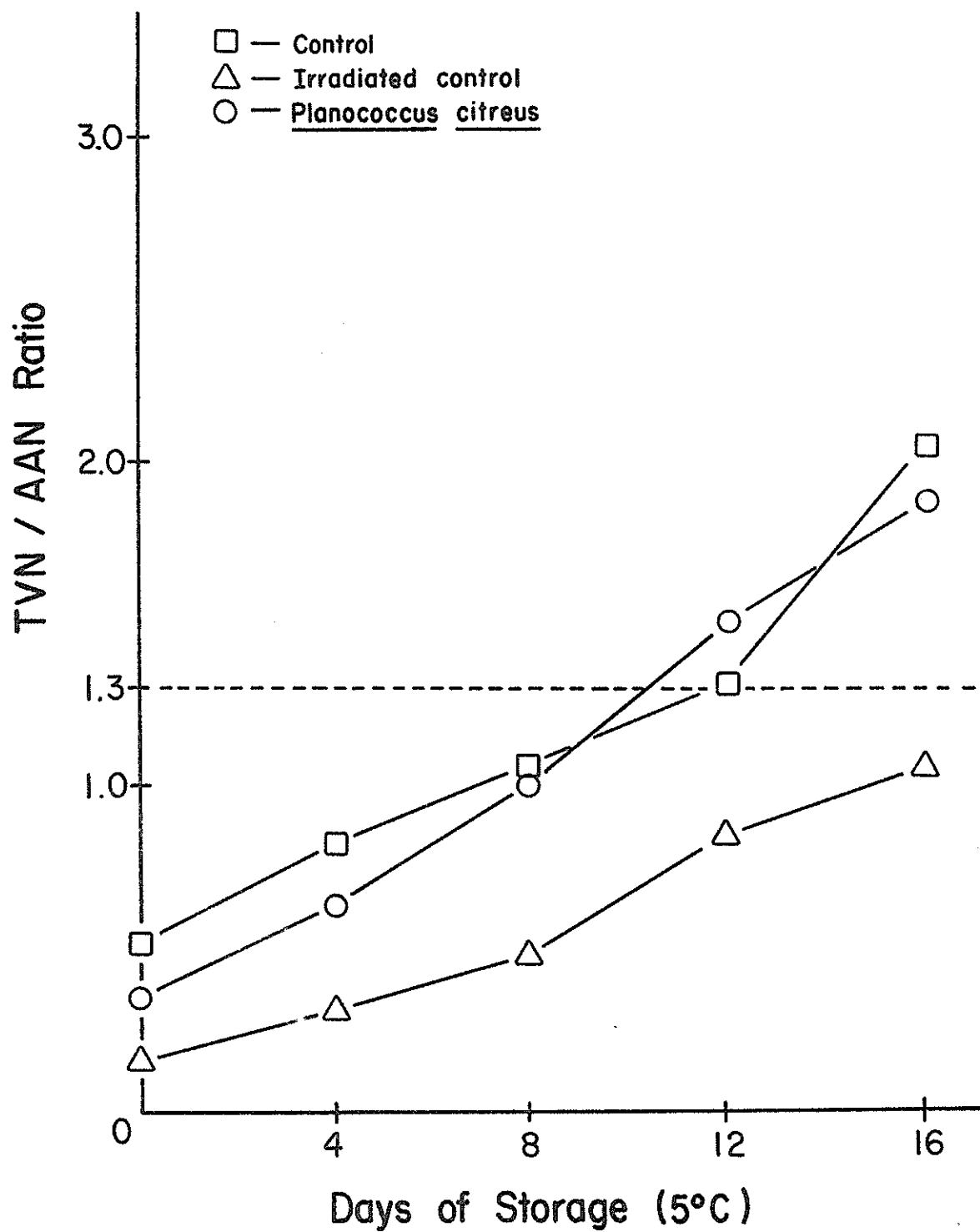


FIGURE 2. CHANGE IN TVN/AAN RATIO IN SHRIMP STORED AT 5°C FOR 16 DAYS.

QUALITY ACCEPTANCE INSPECTION OF SEXUALLY  
MATURE FROZEN FEMALE MULLET

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The United States Department of Commerce, Seafood Quality and Inspection Division has performed export acceptance inspection on sexually mature female mullet and mullet roe. Product is principally exported to Taiwan where a dried lightly salted fish roe product called Karasumi is manufactured. Apparently, processors in the United States do not possess the necessary art in manufacturing Karasumi acceptable to the Asian markets. This may be due to careless handling.

The matter of art is more easily understood when I have found that it has taken us four years of inspecting sexually mature female mullet to determine and define what the quality factors are for product exported to Taiwan. When the Taiwanese speak of quality they may be referring to the weight of the roe rather than to color, texture, uniformity, state of maturity, condition of roe etc. Size is a very important quality factor. Sexually mature female fish caught the first week in December are generally best along the central Gulf Coast of Florida.

Gradually through our four years of experience inspecting millions of pounds of roe mullet and mullet roe, our knowledge and appreciation of the fact that examining product destined to be processed into Karasumi is not only a science but an art has developed through this appreciation of some of the fine points connected with our examinations. I have developed an inspection protocol which is worthy of recording in an organized fashion from the inspection viewpoint. What follows is a protocol somewhat unique to our ordinarily pragmatic approach to inspection.

Karasumi to be of high quality must be processed in a very special way. The Taiwanese and Japanese prize the roe from mullet and particularly like the roe from the striped mullet, Mugil cephalus, commonly called black mullet in Florida. Florida mullet particularly the large fish taken off the central Florida Gulf Coast is well liked as suitable for processing into Karasumi. The larger the mature female mullet roe is, the more acceptable and desirable it is. Good quality Karasumi made from 7½ oz. or above roe may retail for 35 to 45 dollars per pound. Therefore, it is an extraordinarily valuable and expensive product.

Karasumi is manufactured by lightly salting the roe sacs and aligning them on planks covered with rice paper. The roe sacs are covered on top by rice paper and a wooden plank placed on the top with appropriate weights to gently squeeze the roe sacs pressing them into the desired shape and assisting in the drying process.

The roe sacs are pressed at night indoors and are sun dried outdoors during the day on bamboo screens. This process is repeated with nightly pressing and day time sun drying until the moisture content is at the desirable level and the product will not spoil for a considerable time. It is then essentially a dried preserved lightly salted roe product and is named Karasumi.

The purpose of my study was to define and specifically address the quality factors inherent in the sexually mature female mullet which add to or enhances its desirability and value for use in the manufacture of good quality Karasumi. Employment of this inspection and certification protocol will assure that acceptable product is exported to Taiwan, which is our objective.

#### MATERIALS AND METHODS

The method used in developing our inspection protocol was to identify and make observations on inherent factors found in sexually mature female mullet and its roe and in the amount and frequency of variations of these specific factors. Observations were performed with increased frequency and attention as various factors became better identified and became more easily measured. Table one shows the volume of inspected roe mullet and mullet roe for the previous four seasons.

Materials used were the sexually mature female mullet and the roe sacs which had been removed from mature female fish. Work sheets and certificates generated from inspections over the previous four years were used to quantitatively evaluate and establish ranges and limits to fish and fish roe characteristics. The third source of material was the method of questioning individuals in all areas of the business. These were processors, middlemen or wholesalers, exporters, trading companies and buyers. Some material was acquired in written form from purchase orders, sale agreements, bills of lading and letters of credit furnished me from the various individuals engaged in the trade. To my knowledge, none of this information has ever been compiled and formalized before.

Results obtained by the above three methods were fed back into a developing inspectional protocol and efforts were made to see if they were appropriate and if they fit what we were attempting to do. This was the tried and true trial and error method. Evaluation of the success and/or failure of these changes in protocol enabled me to make fine tuning adjustments. Finally, the pieces begin to fit together and a reasonably satisfactory inspection and certification was developed and standardized as part of our inspection procedures.

## RESULTS AND DISCUSSION

Quality factors of fish and roe found during inspection compared with desired quality of buyer.

### Mullet fish

Frozen fish offered for USDC inspection for export were found to possess the following characteristics:

#### Factors

1. Range of weight: 1.4 lbs to 3.0 lbs  
Desirable range: 2.5 to 3 lbs
2. Percent of sexually mature female fish: 93.3% - 100%  
Desirable range: 95% - 100%
3. Weight of fish to weight of female roe found:

<u>Wt of Fish</u>	<u>Percent Roe</u>
2.2 lbs	15.6%
2.2 lbs	17.6%
2.3 lbs	16.8%
2.3 lbs	17.6%
2.3 lbs	18.6%
2.4 lbs	18.0%
2.5 lbs	21.0%
- Desirable weight of female fish: 2½ lbs up  
Desirable percent roe: 17% up
4. Quality of fish: Slight deterioration to good fresh appearance.  
Desirable quality of fish: Prime quality, no deterioration, fish must be hard frozen and frozen within 24 hours.

### Mullet roe

Frozen roe offered for USDC inspection for export were found to possess the following characteristics:

1. Weight of roe found: > 4 oz small  
4-6 oz medium  
6-8 oz large  
Desirable weight of roe: 7½ oz
2. Color found: creamy white  
yellow  
orange  
amber  
Desirable color: yellowish orange to amber

3. Condition found: Multilated to intact.
- Desirable condition: No nicks, cuts, tears, separated sacs, mashed or misshapen sacs.
4. Appearance found: Unequal size of individual roe, shrunken roe, to perfectly formed and full bodied well shaped roe.
- Desirable appearance: Uniformly shaped, plump roe sacs fastened together, membrane intact with one inch of oviduct intact.
5. Defects found: Grayish to blackened areas, purplish bruises, blood marked, dirty unwashed to clean, washed sacs with uniformly of color, no grayish or blackened areas, no bruises or blood marks.
- Desirable: Clean with complete freedom from darkened areas, bruises or blood marks.

Packaging and packing requirements.

#### Mullet fish

Sexually mature fish are packaged with no additives in layers with fish laid head to tail in 25 or 50 pound corrugated fiberboard boxes sealed with glue and banded. Fish may or may not be glazed. Product shipped in unitized containers holding 38 - 40 thousand pounds.

#### Mullet roe

Each roe sac must be packaged in a pliofilm or polyfilm bag or sleeve separately. Bags or sleeves packaged into 3 or 5 pound individual cartons which are packed into 50/60 pound master cases, glued and banded. Master cases are shipped in unitized containers holding 36 - 38 thousand pounds.

Importers in foreign countries, particularly Taiwan, want only large size roe over 6 oz, preferably 7½ oz. Roe must be free from damage such as ruptured membranes or cuts and nicks. Roe sacs must consist of two gonads joined together not separated. Roe must not possess any grayish or blackish discoloration, be free from blood areas on surface of roe, be plump, uniformly shaped and fully mature. Roes must possess color hues from golden yellow to orange-amber and good flavor and odor. Roe sacs must be individually packaged in pliofilm or polyfilm sleeves or bags and placed in waxed cartons to protect from dehydration. Roes must be quick frozen.

Mullet packaged as sexually mature female fish must be over 95% female fish and fish must average over 2.2 pounds, preferably 2½ - 3 pounds. Fish must be prime condition, hard frozen and frozen quickly within 24 hours of harvesting. Ratio of roe weight to weight of fish must be over 15% and preferably 17 - 18%.

#### CONCLUSIONS

Quality criteria levels have been established for mullet and mullet roe. Protocol for inspection of sexually mature female mullet and mullet roe will be performed in accordance with quality criteria given in results of this paper. Certification will be made based upon requirements of buyer if requested by exporter/applicant. If buyers' requirements are not specified, product will be inspected only for factors requested by applicant with a statement to that effect placed upon the body of the official export certificate.

If product is not found to meet buyers' requirements, this will be stated on the certificate and an adjustment or buyer/seller agreement worked out based upon the results of the inspection.

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SEASON	FEMALE MULLET	MULLET ROE
1975 - 76	1,330.8	39.8
1976 - 77	666.8	19.3
1977 - 78	839.6	44.5
1978 - 79	1,650.7	206.8

TABLE 1: Quantity of Female Mullet and Mullet Roe Inspected  
(IN THOUSAND POUNDS)



## FLUID SEPARATION OF FISH BY SHAPE

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Recently, an incentive to develop new techniques in fisheries has evolved (9), especially with the harvest of mixed, under-utilized species becoming increasingly significant (2). Separation of marine food products by shape or size is of particular interest, either by commercial fisheries or aboard ship.

Gillies (6) reported that a rapid and efficient way of separating shrimp from fish which could be used aboard a shrimp boat has been achieved by Lovett. A less labor intensive method of mechanical separation of fresh water prawns into different market grades has been used (12) with a pilot system used for harvesting them. An improved catch-handling method was developed in Denmark in a North Sea trawler for the separation of smaller species from larger species using a rotating caged grader.

The principle of mechanical separation based on the freely moving body in a fluid stream with the imposition of a cross flow of fluid was suggested (11) to achieve such a separation. This principle of fluid separation has been used to separate chaff from grain as in winnowing (3) and in the separation of potatoes from stones (10), to mention but a few applications.

The dependence of density and buoyancy on the physiology of fish has been dealt with (1). A fish (e.g., flounder) which has no special buoyancy organs usually has a specific gravity between 1.06 and 1.09. Others (e.g., whiting) that have buoyancy organs could have a variation in the apparent density on the extent of expansion of the swim bladder. Thus, fish may float or sink and the unreliability of separation in water becomes imminent.

Brown (4) cited data for the maximum velocity of spherical particles of widely varying sizes and densities through fluids, allowing calculation of the pertinent engineering information.

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The shape of a particle as defined in terms of sphericity,  $\Psi$ , is equal to the ratio of the surface area of the sphere to that of a particle of equivalent volume. Sphericity (7) has been one of the most important factors used for the determination of the effect of particle shape on settling velocity.

For two dimensional motion, the balance of forces of a fish projected in air is given by:

$$\frac{mdu_h}{dt} = -F_D \cos \beta \quad \text{in the horizontal direction, and} \quad (1)$$

$$\frac{mdu_v}{dt} = mg \frac{(\rho_f - \rho_a)}{\rho_f} - F_D \sin \beta \quad \text{in the vertical direction.} \quad (2)$$

$$\text{The drag force, } F_D = \frac{1}{2} C_D \rho_a A_p u_r^2$$

$$\text{then } \frac{du_h}{dt} = \frac{C_D \rho_a A_p u_r u_h}{2m} \quad (3)$$

$$\text{and } \frac{du_v}{dt} = g \frac{(\rho_f - \rho_a)}{\rho_f} - \frac{C_D \rho_a A_p u_r u_v}{2m} \quad (4)$$

If fish are discharged at a height,  $h$ , from a belt conveyor moving at a constant speed,  $v_B$ , and inclined at an angle  $\theta$  to the horizontal, the equation of motion in the vertical direction is given by equation (5) neglecting drag.

$$mg - m \frac{d}{dt} (v_B \sin \theta) = -m \frac{d^2 y}{dt^2} \quad (5)$$

from which

$$y = \frac{1}{2} \frac{g}{v_B^2} \sec^2 \theta x^2 + (\tan \theta)x + th \quad (6)$$

If a blower, inclined at angle  $\alpha$  to the horizontal were placed just after the head roll of the belt, a virtual pulse of air would be imparted to a falling fish. The vertical component of the drag force would tend to lift the fish upwards, while the horizontal component would tend to increase its horizontal velocity.

Fish of different shapes have different drag forces depending on their projected area. The trajectory of flat fish, like flounder, is influenced more by the impulse of the air blast than a round

fish such as whiting. Thus, flounder would have a much greater horizontal range compared to whiting, which has a smaller weight per projected area.

#### MATERIALS AND METHODS

The air separation system studied for the separation of flounder and whiting is shown in Figure 1. The components of the system are a conveyor belt, an air blower and a partitioned, water cushioned chamber.

The conveyor belt consisted of a 12-inch wide rubber-coated canvas belt passing over a head and tail roll powered by a  $\frac{1}{4}$  H.P. motor through a variable pitch v-belt pulley, with variable speeds and a chain and sprocket drive. The conveyor frame was supported by four adjustable legs so that the height and angle of the belt conveyor could be controlled. The belt was inclined at an angle of  $5^{\circ}$ . Belt speeds were varied at 0.98, 1.6 and 2.17 m/s.

The centrifugal air blower was powered by a 3 phase, 3 H.P. motor. The diameter of the blower blade was 33 cm (13 inches) with an inlet diameter of 15 cm (6 inches) and an outlet diameter of 13 cm (5 inches). The inlet area opening could be controlled by a throttle plate which was pivoted to cover part of the inlet area.

The outlet from the blower was fitted with a sheet metal nozzle to form a transition from 5 inch diameter to a rectangular cross section with width to match that of the belt. The blower nozzle was placed as near as possible to the discharge of the belt with air directed upward at a  $45^{\circ}$  angle.

A deceleration chamber was placed at a distance from the air blower to collect fish separated by action of the air stream. The chamber, which was essentially a shallow open plywood box, 210 cm x 124 cm x 15 cm, had a partition placed at a distance of 165 cm from the shaft of the blower motor. The inner surface of the chamber was lined with a plastic sheet, enabling water to remain in the chamber to cushion the impact of falling fish.

Each flounder or whiting was hand placed singly on the center of the belt moving at a controlled speed, precisely oriented as described later. Air was controlled to prescribed velocities by adjustment of the throttle plate. Horizontal distances of travel for fish were measured from the motor of the air blower to the point of impact. The belt speed was determined by recording the time taken for a point on the belt to move a measured distance. The variables studied were: Belt speed, air velocity and fish orientation.

Three levels of belt speed were used: 0.98, 1.60 and 2.17 m/s. This determined the initial horizontal component of fish velocity as well as the time for impulse of the force imparted by the air blast.

Air velocity was instrumental in determining the drag force imparted to a fish with two velocities studied: 35.6 and 42.4 m/s.

Fish orientation was with the nose-tail axis (1) parallel and (2) perpendicular to the direction of belt travel.

Before the fish were subjected to separation, mass was recorded as well as observations of horizontal distance of travel of fish.

The trajectories of a flounder and a whiting at air velocity of 42.4 m/s and different belt speeds and orientations were filmed during flight by high speed motion photography. The speed of the film was 64 frames/sec. The TRI-X film was analyzed frame-by-frame using a stop action projector (manufactured by Kodak, 16 mm, Analyst II) at slow speed. The trajectories of these fish are shown in Figure 2.

#### RESULTS AND DISCUSSION

Separation effectiveness was evaluated by the mean difference of horizontal travel of fish after becoming air borne until reaching ground level. Note that the average mass of flounder was greater than that of whiting. Rankings were based on decreasing separation effectiveness. The results of air separation of flounder and whiting are shown in Table 1. The effect of fish mass versus horizontal travel under similar air velocity and belt speed but with different fish orientation w.r.t. the belt are shown in Figures 3 and 4. Complete separation was observed with parallel orientation.

#### CONCLUSION

This investigation was directed toward understanding and exploring the potential application of air separation for two species of fish, flounder and whiting. As an initial step, the use of water as a medium for separation was investigated.

The results can be summarized as follows:

For water separation system:

1. The actual orientation of a fish when it entered the horizontal water stream had a direct bearing as to whether it sank or whether it skipped over the surface.
2. In hydraulic separation the specific gravity of a fish apparently had a negative influence on the desired separation because of variations in swim bladder volume and its effect was more pronounced than sphericity of fish.
3. For good separation of different species of fish it was necessary to reduce the influence of the terminal velocity and this could be achieved by increasing the horizontal velocity of water.

Table 1. Air Separation of Flounder and Whiting.

n = population size = 25

Air Velocity of Blower	Belt Speed	Nose-Tail Axis of Fish Orientation w.r.t. Belt Axis	Horizontal Travel When Fish Reached Ground			Separation Effectiveness	Standard Error of Mean Difference
			Flounder Mean S.D.	Whiting Mean S.D.	Ranking ( $\bar{X}_F - \bar{X}_W$ )		
$v_a$ m/s	$v_B$ m/s		$\bar{X}_F$	$\bar{X}_W$	$s_d = \sqrt{\frac{s_F^2}{n} + \frac{s_W^2}{n}}$		
42.4	0.98	Parallel	278	125	12	1	12
		Perpendicular	179	122	14	8	7
	1.60	Parallel	243	142	10	3	11
		Perpendicular	165	142	10	11	5
	2.17	Parallel	226	146	12	4	6
		Perpendicular	179	140	10	9	5
35.6	0.98	Parallel	219	106	6	2	7
		Perpendicular	146	92	9	7	5
	1.60	Parallel	198	123	5	5	7
		Perpendicular	139	122	8	12	3
	2.17	Parallel	192	122	4	6	4
		Perpendicular	150	118	6	10	3

4. Hydraulic separation necessitated the use of large quantities of water. Sanitation problems were encountered mainly due to fouling of water caused by tearing of skin in the anal region.

5. Hydraulic separation was ruled out as a useful method.

For air separation system:

6. Specific gravity seemed to exert less significant influence as compared to the weight of the fish per unit area. Thus, it was possible to utilize the differences in shape factor to achieve maximum possible separation of the different species of fish.

7. Better control of belt speed and air velocity was achieved as compared with less reliable control of horizontal water velocity used in hydraulic separation. More nearly standard conditions were maintained in air separation facilitating better comparison of results.

8. High velocity imposed by the air blower did not cause appreciable or discernible damage to fish. A well cushioned chamber for collection of separated fish is significant in minimizing damage of fish.

9. Notwithstanding the loud noise of the blower which may have been irritating, the power expended in the air separation device was more than that for pumping water in hydraulic separation.

In conclusion, separation of flounder and whiting can be effectively accomplished with a simple belt conveyor in combination with an air blast at 45°. Fish must be oriented with nose-tail axis parallel to the direction of belt travel. Also, fish must be distributed onto the conveyor in a single layer so that interference of one fish to another is reduced to a minimum. Pilot studies are underway to verify the commercial feasibility of the system.

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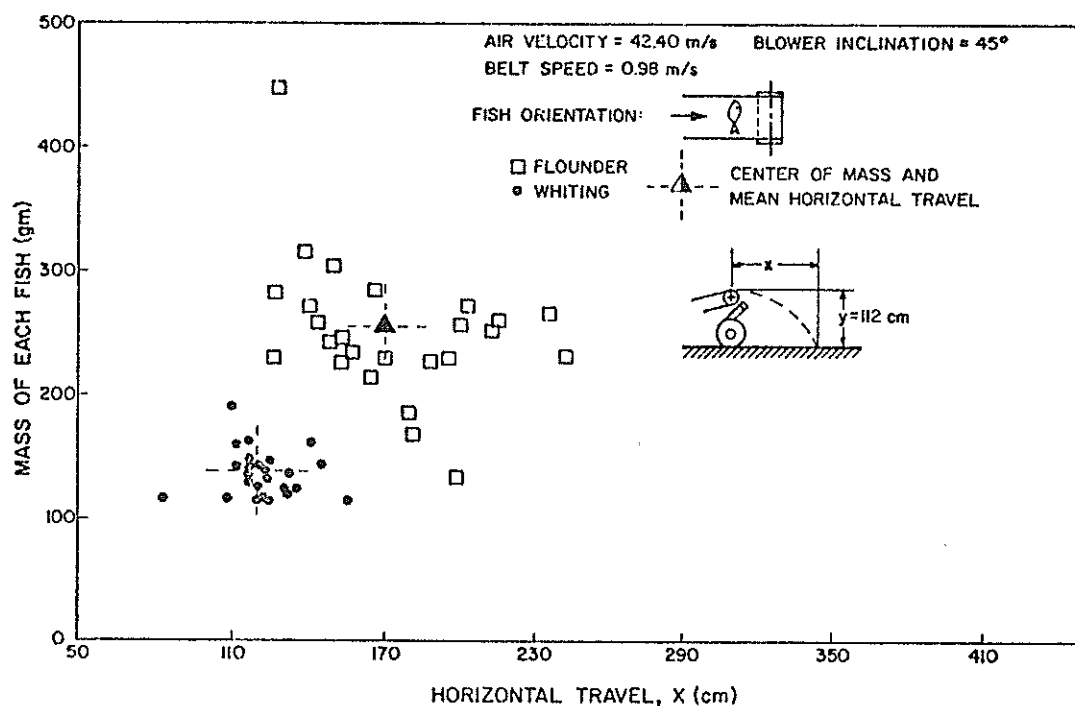


Figure 3. Horizontal travel of individual fish as affected by 42.4 m/s air velocity, 0.98 m/s belt speed, and perpendicular orientation of fish.

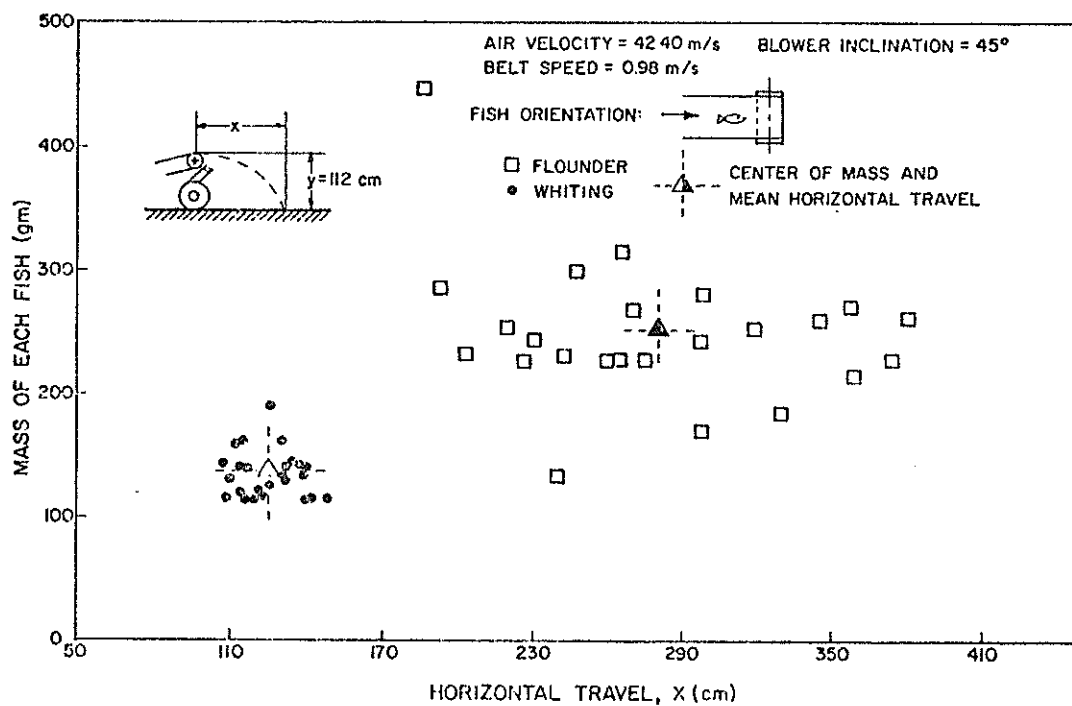


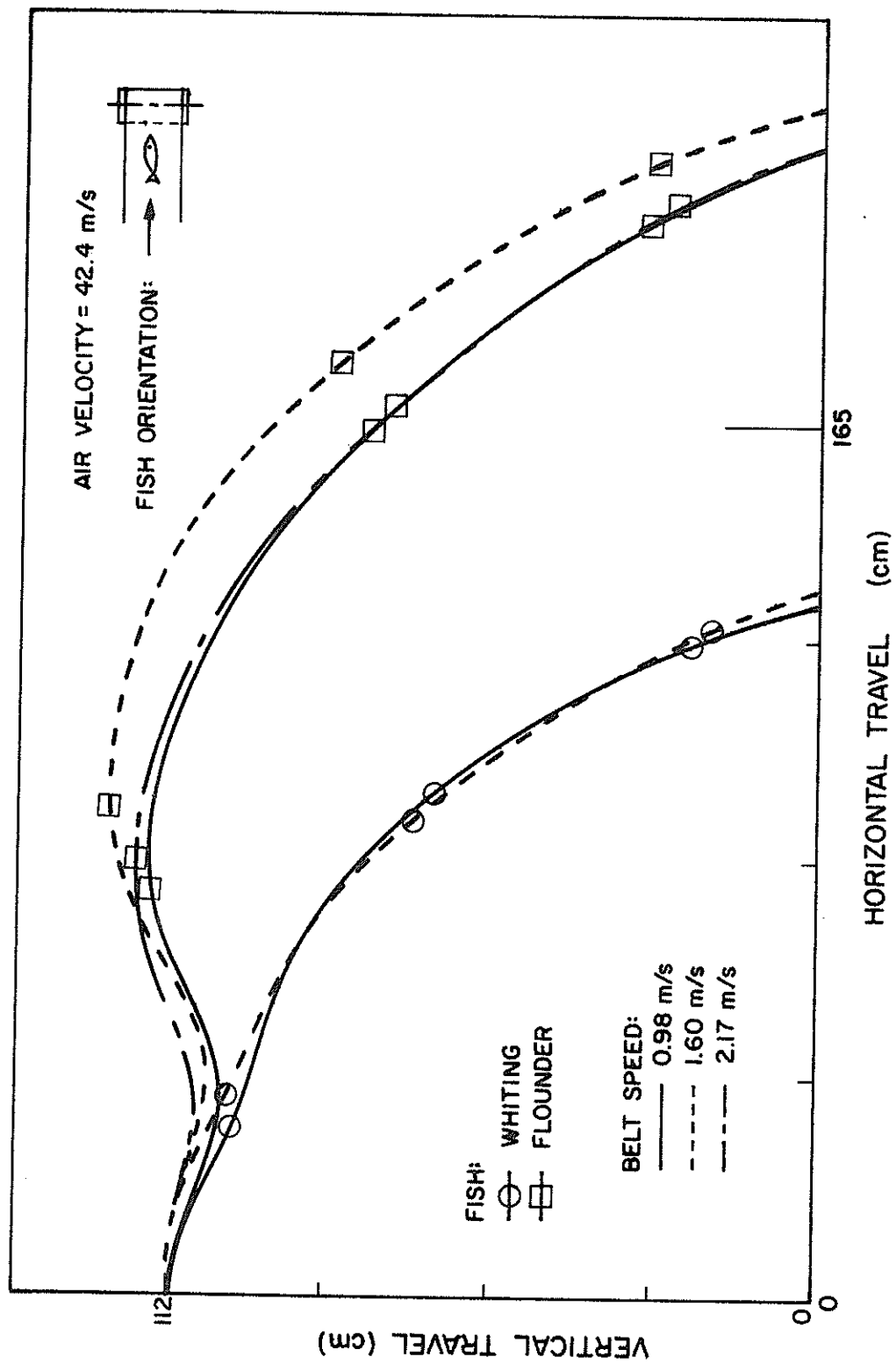
Figure 4. Horizontal travel of individual fish as affected by 42.4 m/s air velocity, 0.98 m/s belt speed and parallel orientation of fish.

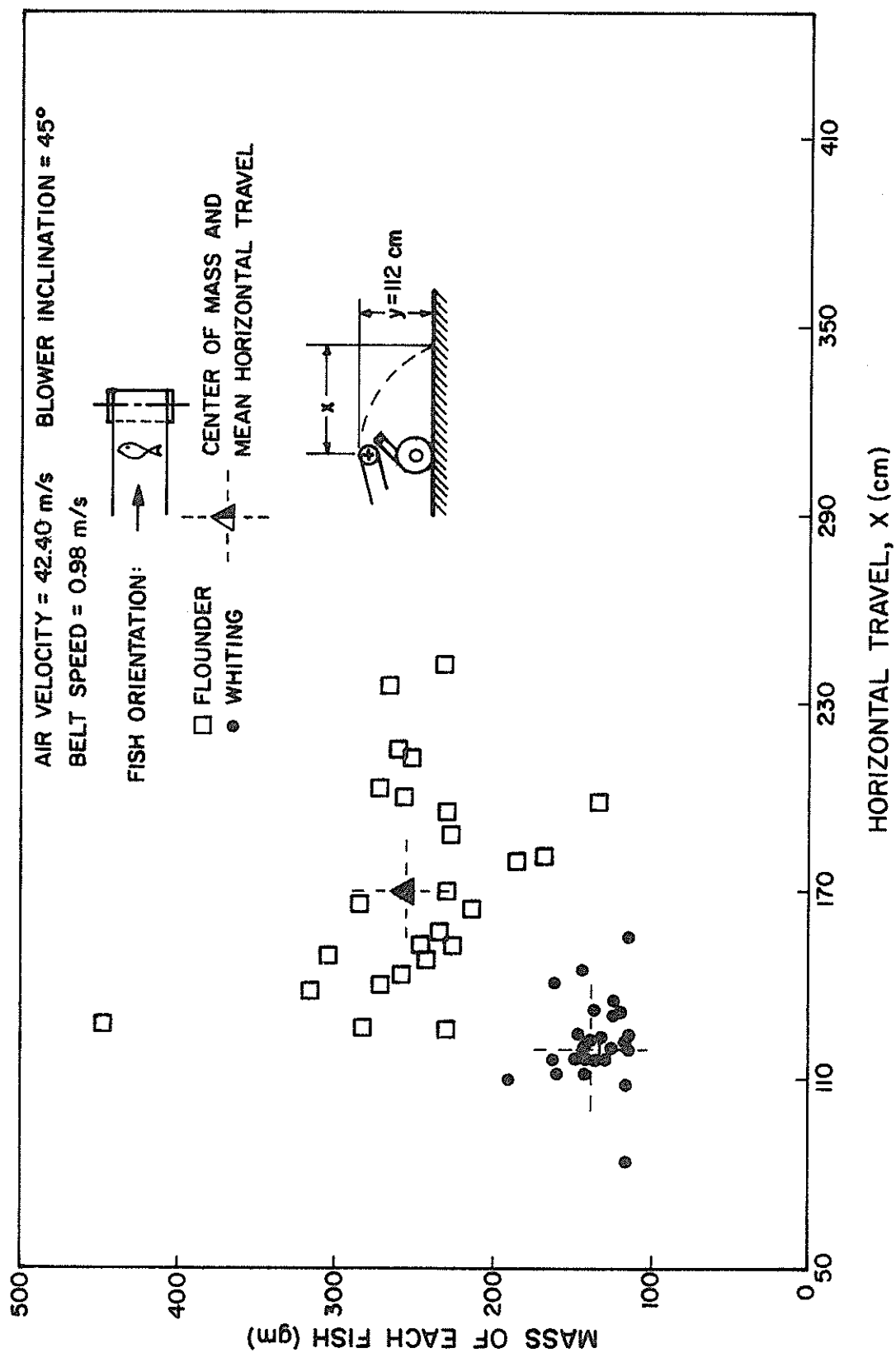
Figure 1. Schematic of air separation device.

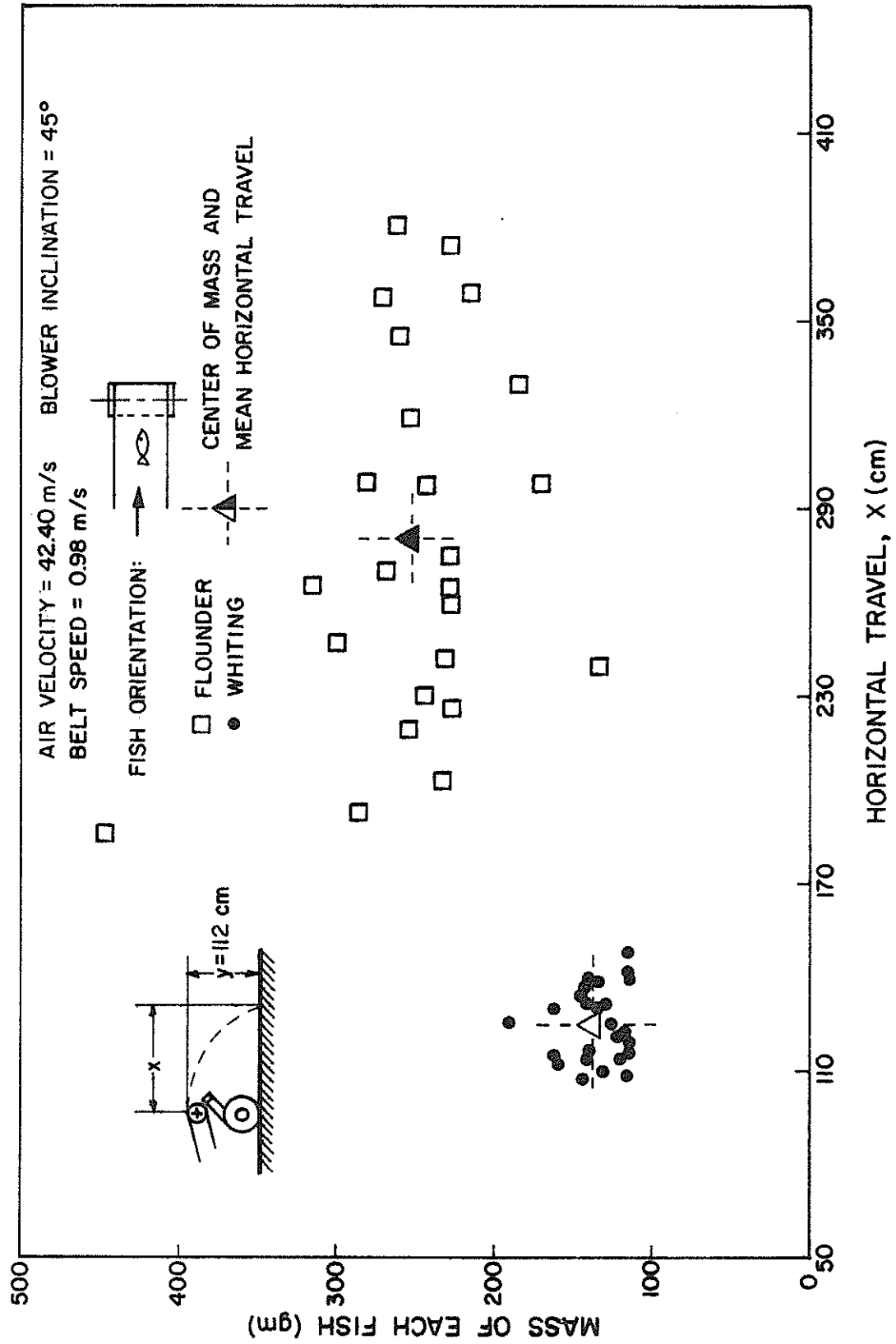
Figure 2. Trajectories of a whiting and a flounder as determined from frame-by-frame analysis of slow motion photography with parallel fish orientation.

Figure 3. Horizontal travel of individual fish as affected by 42.4 m/s air velocity, 0.98 m/s belt speed, and perpendicular orientation of fish.

Figure 4. Horizontal travel of individual fish as affected by 42.4 m/s air velocity, 0.98 m/s belt speed and parallel orientation of fish.







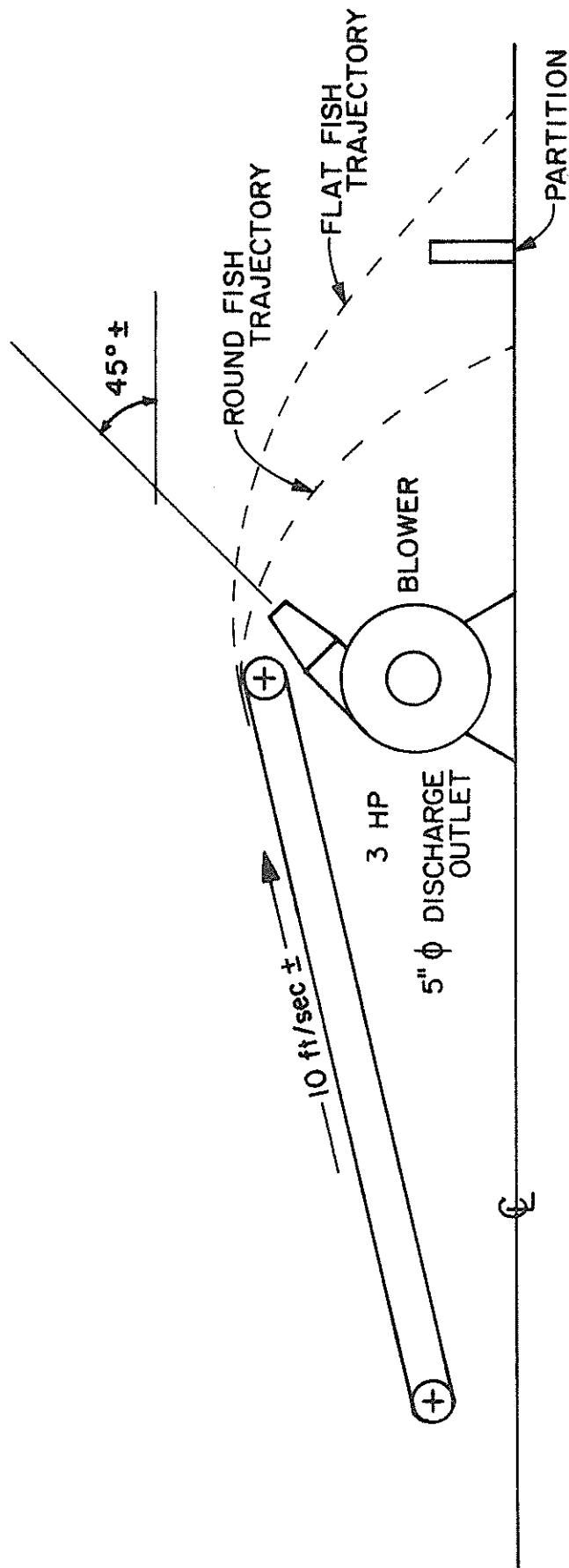


FIGURE 5

# SCHEMATIC OF AIR SEPARATION DEVICE

## THE ECONOMIC FREEZING OF FISH IN A PLATE FREEZER

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Direct contact, plate-type freezers are basically composed of an insulated cabinet which holds freezing plates positioned within a steel frame one above the other like a deck of cards. The plates can be raised and lowered by means of a hydraulic piston or a screw jack. Refrigerant, circulating through the plates, enables them to rapidly cool and freeze the product. The product to be frozen needs to be of a regular size and is therefore usually packed either in a metal tray or in the package in which it is to be sold. The unfrozen product is placed on the plates, after which the plates are lowered. This creates intimate contact between the product and the plates on both top and bottom of the package. Generally, big round fish like tuna or such like, because of their irregular sizes, cannot be frozen economically in a plate freezer unless they are sorted to size and positioned for maximum loading. A blast freezer is a better choice for such fish.

Because the product is in contact with the refrigerated plates, either directly or through a thin package, the heat transfer is attained almost exclusively by conduction. There are no other agents involved (such as air in the case of a blast freezer) and weight loss is therefore zero. After the fish is frozen in the plate freezer, it will be observed that all packages are of exactly uniform size. This greatly increases customer appeal and also facilitates easy packing into bigger cartons and the stacking of those cartons on pallets. The fish is packed into the packages in the soft (unfrozen) state and therefore a dense, economical pack is obtained.

### TYPES OF PLATE FREEZERS

There are basically two broad divisions in types of plate freezers, that is: the horizontal plate freezer and the vertical plate freezer. The latter type is used only for freezing of round fish at sea. The fish is placed by hand or by means of chutes into the pockets formed by the vertical plates and is then frozen. It is therefore fairly easy to load the fish into the plate freezer which can be an advantage on a rolling trawler at sea. The density of the block, however, is not very uniform. These blocks can therefore only

be used for thawing and processing at a land-based factory and may not be suitable for direct sale to customers.

The horizontal plate freezer is used far more extensively and comes in various forms. The smaller units have a self contained refrigeration package mounted in the bottom part of the cabinet, and the freezer plates are situated in the upper portion. These units are extremely economical for the small fish processor. They require very little installation work at site. All that is needed is connecting to the electrical system and a water connection has to be made to the water cooled condenser. Everything else is pre-wired, pre-piped and connected in the unit itself and no further work in the field is required (Fig. 1). One such unit, the Amerio Model 12, can freeze 420 lbs. of shrimp per load, when packed in 5 lb. standard shrimp boxes. Freezing time would be approximately 3½ hours. This means that two loads can easily be frozen in an 8 hour working day and many operators load up the plate freezer in the evening before going home, so that a third load of shrimp can freeze overnight.

For larger freezing capacities, the plate freezers usually have remotely situated refrigeration plants. In many cases these refrigeration plants not only serve the plate freezers, but also serve the other refrigeration duties such as cold storage, ice making equipment, etc. Regarding the capacities of these units, if we stay with our example of the standard 5 lb. shrimp box, then the smaller units in this range will hold from say 2,000 lbs. of shrimp per load, up to about double that quantity for the bigger models.

For the big operators there are the automatic plate freezers. These units may have capacities from 2 tons of product per hour and upwards. The product is fed to these units on a conveyor belt in a continuous stream and pushed automatically into the freezer unit where it is frozen. Discharge is also fully automatic.

#### TYPES OF REFRIGERANTS

Most plate freezers commercially available can be equipped for use with ammonia or for any of the commonly used halocarbons (often referred to by the name Freon, a DuPont trade name). The most commonly used halocarbons are R-12, R-22 and R-502.

Ammonia, also called R-717 or  $\text{NH}_3$ , is the oldest refrigerant still in use today. Maybe for this reason some people tend to consider ammonia "old-fashioned" or not so very efficient. This is totally wrong as ammonia is one of the best and most popular refrigerants in use today for industrial installations.

The halocarbons come into their own for the smaller installations. The small self contained plate freezer with the compressor unit mounted in the bottom of the cabinet invariably uses halocarbons. Plate freezers for halocarbons usually use thermal expansion valves (one on each plate) to control the refrigerant. Some units have been built for recirculated R-22 operation.



## CONSTRUCTION OF PLATE FREEZERS

The manual type double contact plate freezers basically consist of an insulated box in which the freezer plates are fitted. The insulated box can have a stainless steel, galvanized or glass-fiber outer and inner cladding with insulation fitted in between. Doors with special hardware and sealing gaskets are fitted in the front as well as at the back of the cabinet. The plates are stacked horizontally in the cabinet and they can be moved up and down by means of a hydraulic system. When the freezer is ready to be loaded with product, the plates are raised in the upper position by means of a hydraulic cylinder. The packaged fish is then positioned on the freezing plates and when the plate freezer is fully loaded, the plates are lowered again hydraulically. The freezing cycle can now commence after the doors have been closed. The refrigerant is fed from a stationary liquid header through special flexible hoses into the one end of the freezer plates. The refrigerant absorbs heat from the fish to be frozen and thereby flashes into a gas. This gas is removed on the other side of the plate, again through a flexible hose into a suction header and from there removed by the refrigeration compressor. It must be pointed out here that the hoses are of a special type, suitable for the refrigerant being used. Replacing of these hoses by other makes and types not suitable for refrigeration duties can lead to extremely dangerous situations.

### TYPES OF PLATES

Almost all manufacturers of plate freezers used to use steel to manufacture their plates. These steel plates (sometimes also called vacuum plates) consist of a top and bottom skin of sheet metal in between which a single or double serpentine coil is situated. These coils are usually made of square tubing and a vacuum is drawn between the plates to ensure good contact between the serpentine coil and the metal skins. The outside of these plates is zinc sprayed to stop corrosion. The refrigerant enters the coil on the one side and, as explained earlier, extracts the heat from the product to be frozen. This heat is conducted through the walls of the coil and through the upper and lower skin of the freezer plate. The results obtained with these plates are good, although they have limitation in the case of the bigger plate sizes as used in the automatic plate freezers.

A later development is the use of extruded aluminum to fabricate the freezer plates. Although aluminum is considerably more expensive, the use of this material for freezer plates has a number of advantages. By use of parallel and progressively expanding refrigeration circuiting in the plates, a far better and more uniform flow of refrigerant through the plates is obtained. This results in a very uniform plate temperature, and an extremely efficient refrigeration circuit within the plates. Also due to the particular construction of the aluminum plates, a far more intimate, direct contact is possible between the refrigerant and the product to be frozen. In fact, the contact area between refrigerant and product is almost 99% of the total plate area. In addition, the aluminum plates are considerably stronger. As you know, equipment in the fish processing industry

is subjected to a lot of abuse and rough handling. The aluminum will last considerably longer in this type of environment than the steel vacuum plates would. Our company manufactures the well known Amerio plate freezers, which can be fitted with either steel plates or aluminum plates. Although the lower cost of the steel plates may be tempting for a potential buyer, the aluminum plates should be considered as a better investment. Not only will they last much longer, but they provide shorter freezing times due to the superior refrigeration circuiting and better transfer of heat from the product to refrigerant.

Plate freezers are generally custom built for a certain package thickness. The 5 lb. shrimp box in our previous example has a thickness of 2-3/4 inches. An Amerio plate freezer with 16 stations would be selected for this product. This unit has a station opening which is instantly variable from 1-1/8 inch minimum to 3-1/4 inches maximum. Allowing for clearance, this unit will accept package thicknesses from say 3 inches to 1-1/8 inch.

#### CONCLUSION

Plate freezers provide one of the most economical ways to freeze packaged fish products. Intimate contact between refrigerated plates and product ensures rapid freezing, resulting in high quality, uniform products. Weight losses are zero. Plate freezers require less refrigeration, use less power and occupy less space than conventional blast freezers.

## THE CENTRIFUMATIC CRAB MACHINE

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The spiraling costs of labor coupled with the attrition of experienced hand-pickers, and the reluctance of younger people to enter into this vocation, has resulted in large increases in both the cost of producing crab meat and amount of bone content therein. At the present time most of the 250-350 million "shole crab" pounds caught nationally is hand-picked. Efforts to pick crabs mechanically extend back to the 1920's, but have not made enough impact on the industry to cause any trend away from hand-picking techniques.

A review of the above salient facts indicates a great need to design and build a mechanized crab-picking device that will economically extract crab meat from crab sections, retaining the taste and physical characteristics of hand-picked meat, while maintaining a low bacteria count and minimal bone content.

### OPERATIONS

The Centrifumatic Crab Machine was developed to replace the need for skilled handpickers, and allow processors more rigid control over the product they produce. It's design is such as to allow automatic removal of meat from various sizes of crab sections, without adjustments to machine or grading of "whole crab", in a safe, sanitary manner, and with a minimum of bone content.

The machine consists of these three integral operations:

- a. Cooking Retort
- b. Coring Machine
- c. Extractor

It is a continuous in-line operation starting with the crabs being cooked in retorts for a specified time, with controlled temperature and steam pressure. The crabs are removed from the retort, or if pre-cooked from a refrigerated area, to a stainless steel table (which makes for convenient loading by an operator). The conveyor carries the crab through a saw that removes the mouth and eyes section, permitting the cut section to drop into a container. The crab is then conveyed to the Coring Machine, aligned on a centering rod, and conveyed to a debacking device that removes the outer shell. In sequence, the claws, flippers, lungs, and viscera are removed into waste containers, and the cleaned core is conveyed through a dual-saw combination that cuts out the center bone structure and divides the core into two halves. This allows one face on each half core to be opened so meat can be extracted. With the center bone section now removed the half-core sections are inspected for any extraneous or foreign materials and placed in a stainless pan convenient for loading the continuously moving Extractor spinners.

Each of these spinner units have two core receiving receptacles placed 180° opposite to each other. At the loading station the Spinner-Extractors are indexed in a stationary position that allows quick and easy loading by the operator. This loading operation serves one other function--which is that of closing the portholes for each receptacle so extracted crab meat is retained in receptacles until rejected at a timed sequence.

The loaded spinners are conveyed to a section of the machine where centrifugal force is developed by applied rotation. This removes the crab meat from the cores and compacts the backfin and other meat into a lump configuration in the extremity of the receivers. The extractors are now subjected to a braking application which causes the spinner rotation to stop--and allows the spinner to be indexed so meat ejection opening in bottom of receiver is aligned directly over synchronized moving shipping containers. The containers are now positioned to receive the meat which is ejected by means of a plunger unit. With the meat in the container, the container conveyor moves to a location where the operator can inspect and remove the filled containers for weighing and replacing them with empty containers. These containers can vary in size from 8, 12, and 16 oz.

The conveyor carrying the spinner extractor continues its cycle to the next operation where the empty cores are dropped into waiting collector units. The conveyor -- with extractors having completed the productive portion of the cycle -- returns back to the 1 loading station. Enroute the spinners are thoroughly washed with three separate high pressure water nozzles which sanitizes each receptacle prior to its new productive cycle.

Contributing factors to this being a low bacteria plate count operation are threefold:

1. The speed with which the crabs are processed (60 pounds) minimizes the time element necessary to increase the plate count; adjustments can be added to increase the production rate.
2. The sanitation procedures incorporated into the design of the machine which removes any crab residual.
3. The method of handling only the shell portions of the crabs, and not the meat sections, eliminates the potential contamination by hands in the processing.

#### CONCLUSIONS

The introduction of the Centrifumatic Crab Machine to the crab industry permits the use of non-experienced personnel with no resultant increase in shell and bone content.

The mechanical method of crab-picking, where a small number of employees are involved, allows for greater latitude in coping with problems created by the inconsistent quantities of crabs that are available on a day-to-day basis.

As more rigid controls become more of a trend by those regulatory forces controlling health and sanitation regulations, there will be created a strong demand for improved methods of production which will favor the use of mechanical pickers.

## SOLAR DRYING OF SEAFOOD PRODUCTS: MULLET ROE

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Solar drying was one of the earliest known techniques of food preservation and is still practiced in the developing nations where high imported fuel costs limit the use of more controlled conditions which can be obtained using natural gas fired heated air units. Recent increases in the price of fossil fuel coupled with the certainty of further price increases have led to a resumption of interest in solar drying.

The state of Florida is an area which offers a good probability of success in solar drying. Direct normal insolation (12 noon at 28°N latitude) ranges from 275 BTU/ft<sup>2</sup>/hr in February to 255 BTU/ft<sup>2</sup>/hr in June (2). Open sun drying, as traditionally practiced, is unsuitable since periods of peak insolation (incoming solar radiation) generally coincide with periods of rainfall. The unprotected dried product can then absorb moisture with a corresponding decrease in drying rate and increased likelihood of mold damage. Insect damage due to flies is also a particular problem (7).

Fish drying in the U.S. and Canada dates back to the Massachusetts Bay Colony (1,15). Primarily cod is salted and dried in the open on drying racks to form either dried cod or lutefisk. Production is relatively large (2,000,000 lbs in 1970 for lutefisk) (16) but brings a relatively low price (37¢/lb). Other products dried in the open sun include tuna (89 778#) and shrimp 815 111#) in addition to such specialty items as shark fin and octopus.

In contrast, throughout the Orient, dried fish is a staple of the diet and comprises a sizeable industry (15). Products dried in major quantities include squid (1968 production of 27,000 tons), sardine (52,371 tons) and shrimp (3,798 tons). Fish roe is also an important product (24,700 and 2,060 tons for cod and herring roe).

Mullet roe in particular is a product which is dried using solar energy and processing techniques altered to give a good quality product using solar drying methods. Large quantities of roe are produced (2 million lbs in 1973) (6) and salt cured to form products such as bottarga, batrakh and karasumi (12). The large price differential between the raw and finished product (\$1.50 vs \$10-\$50/lb for the finished karasumi) makes it advantageous to duplicate the process and then to export the finished product.

Three types of solar driers are used including direct insolation, forced heated air and combination direct insolation-forced heated air (3,13,14). In direct insolation, the product itself absorbs the solar radiation elevating the temperature beyond the surrounding air (13). Unlike open sun drying, the product is protected from the environment and damage by insects. Forced heated air driers require external collectors with the air entering the drying chamber either through natural convection or by use of blowers. Combination type units use both external collectors and transparent cabinets to permit more rapid drying than forced heated air units while still permitting a greater extent of control over drying than the direct insolation unit alone.

In direct insolation drying, sunlight is absorbed by the product leading to an increase in moisture vapor pressure. Moisture then flows from the region of high vapor pressure (inside the product) to regions of lower vapor pressure (the air outside the product). In this type of unit, increasing the air flow past the product would be expected to cool the product leading to a reduction in drying rate rather than to an increased drying rate as would be expected in a conventional forced heated air unit (8).

In these studies, preliminary studies on the effect of varying air flow rates on the drying rates and product temperatures of mullet roe in model direct insolation driers was examined with the goal of further improving the drying system.

## MATERIAL AND METHODS

### Curing of roe

Mullet roe was prepared using the method of Hsu (9) where thawed roe was dry salted on a 15% wet basis and cured at 35°F for 8.4h. After desalting for 6h the cured desalted roes were used for drying.

### Direct insolation driers

Four different driers were constructed to examine the effects of different air flow directions on the drying rate of mullet roe (Fig. 1). Small blowers having approximate air flow rates of 15 ft<sup>3</sup>/min were installed to give either side to side or bottom to top air flow. In natural convection units, the appropriate sides were screened and left open to the outside air. In later trials, blowers having higher air flow rates were installed giving bottom-to-top air flow patterns (Fig. 2).

The unit itself consists of a wooden chamber of dimensions 36" x 24" x 6" with a plywood base. The product is placed on wooden trays consisting of overlapping polyurethane treated cedar boards through which 3/8" holes are drilled to allow sufficient air flow from beneath the product. As in the traditional method

of the Japanese, the product was pressed between two trays overnight to improve both moisture distribution and product appearance (7). A translucent sheet of fiberglass served to cover the unit, trapping the heated air and preventing rain entry (Figs. 1 and 2).

### Drying tests

Prepared samples were placed on product trays and 36 gauge copper-constantin thermocouples placed within the product to measure product temperatures. Air temperatures were made using shielded thermocouples placed near the air inlet and outlet and data recorded at 15 or 20 minute intervals using a data acquisition system. Air flow measurements were taken at the blower inlet and drying rates measured by daily product weighings. Insolation was measured using an Eppley pyranometer channelled into the data acquisition system.

## RESULTS AND DISCUSSION

Little difference in drying rate between natural and forced ventilation was observed between either side to side or bottom to top air flow patterns. A falling rate drying curve was observed. This is probably due to the roe membrane which can restrict water transfer (10). A 20% dry basis moisture content was reached after an 8 day drying period.

Later tests were conducted using forced ventilation units with various inlet restrictions to avoid difficulties involved in measuring air flows for natural convection units. Air flow rates were adjusted by restricting inlet openings and measured with a differential pressure type air flow element.

In these tests conducted September 16 to 20, 1978, differences in drying rate were apparent (Fig. 3). The most rapid drying rate was observed for the forced ventilation unit having a 17.3 ft<sup>3</sup>/min air flow (-.-.-) followed by natural convection (-----). Increasing the air flow led to a reduction in drying rates with the product reaching a 20% dry basis moisture content after a four day drying period (Fig. 3).

This reduction of product temperature with an increase in air flow is reflected in Table 1. Product temperatures reached 152°F for natural convection units while increasing air flow led to a reduction in product temperature. Both the natural and forced ventilation units (17.3 ft<sup>3</sup>/min) have air flow rates of the same order of magnitude as reflected by similar product and air temperatures.

Browning, due both to elevated product temperatures and high roe lipid and protein content, was observed to proceed from the ends of the roe toward the center. Ideally, the product should be medium orange brown in color with excessive browning leading to a poorer



color and consequent reduction in price (15). After four days of drying the darker roe have begun to blacken. However, little difference in the extent of browning was observed between the driers indicating that temperatures within the cabinet were excessive.

Due to the high protein and lipid concentrations of the roe (9) Maillard browning and lipid oxidation can occur with the browning rate reaching a maximum at a 15 to 20% moisture level. In this case, the product temperature is sufficiently high that extensive browning and protein denaturation can take place (7).

#### CONCLUSION

The results presented here show there is a relation between drying rate and product temperatures with increased air flows cooling the product and reducing the drying rate. Extensive browning was observed indicating that reduced product temperatures are necessary to minimize the extent of lipid oxidation and Maillard browning.

To accomplish this reduction, the simplest method with the greatest likelihood of success is to simply increase the size of the dryer and to construct a greenhouse-type structure. Lower air temperatures result with increased heat transfer from the heated product to the cooler air. Reduction in drying rates would lead to lessening of case hardening due to more uniform moisture removal throughout the drying period.

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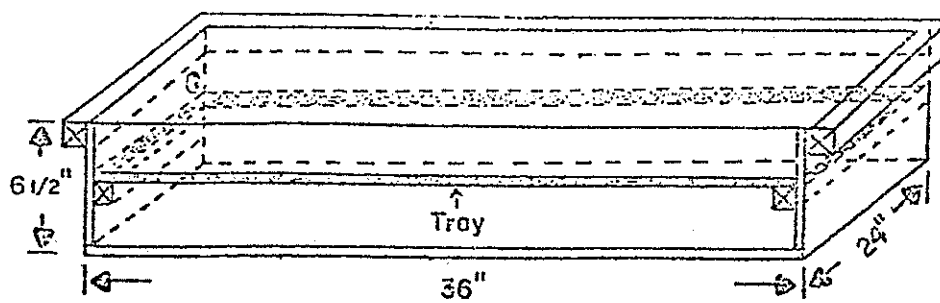
TABLE 1. Maximum Product Temperatures (°F) Sept. 16-20, 1978

<u>Air Flow (ft<sup>3</sup>/min)</u>	<u>Air Out</u>	<u>Product</u>	<u>Ambient</u>	<u>ΔT Prod.</u>	<u>ΔT Air</u>
43.36	115.5	132.1	89.4	42.7	26.0
17.29	130.7	148.3	89.4	58.9	41.3
50.28	116.8	127.4	89.4	38.0	27.4
natural convection	143.1	151.3	89.4	61.8	53.7

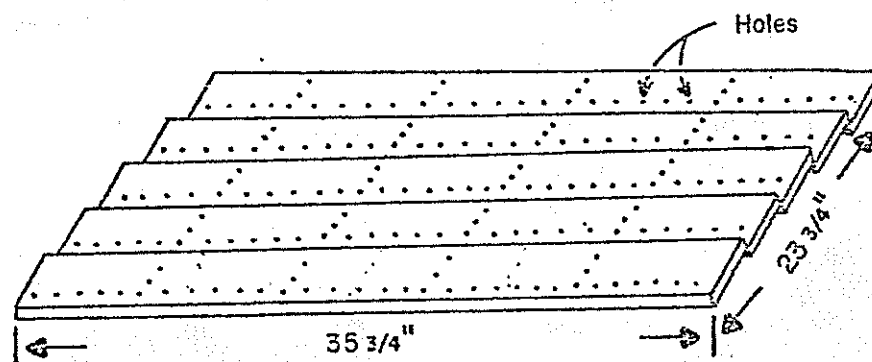
Peak insolation: 127.2 BTU/hr/ft<sup>2</sup>

Air flow (forced ventilation): variable

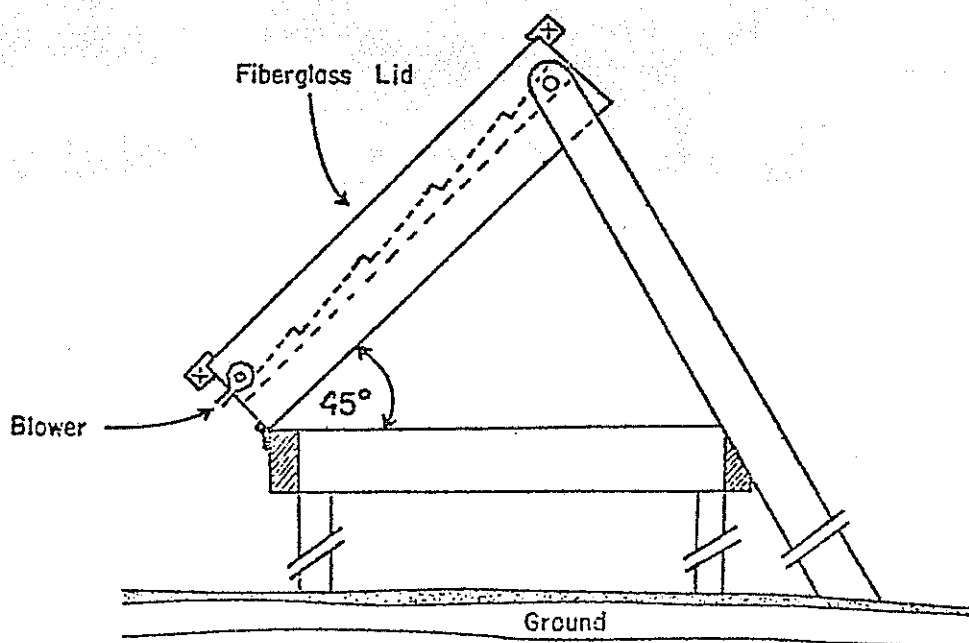
Air flow direction: bottom to top



Drying Cabinet



Product Tray



Drying Cabinet Mounted

FIG. 1 DIRECT SOLAR INSOLATION DRYER

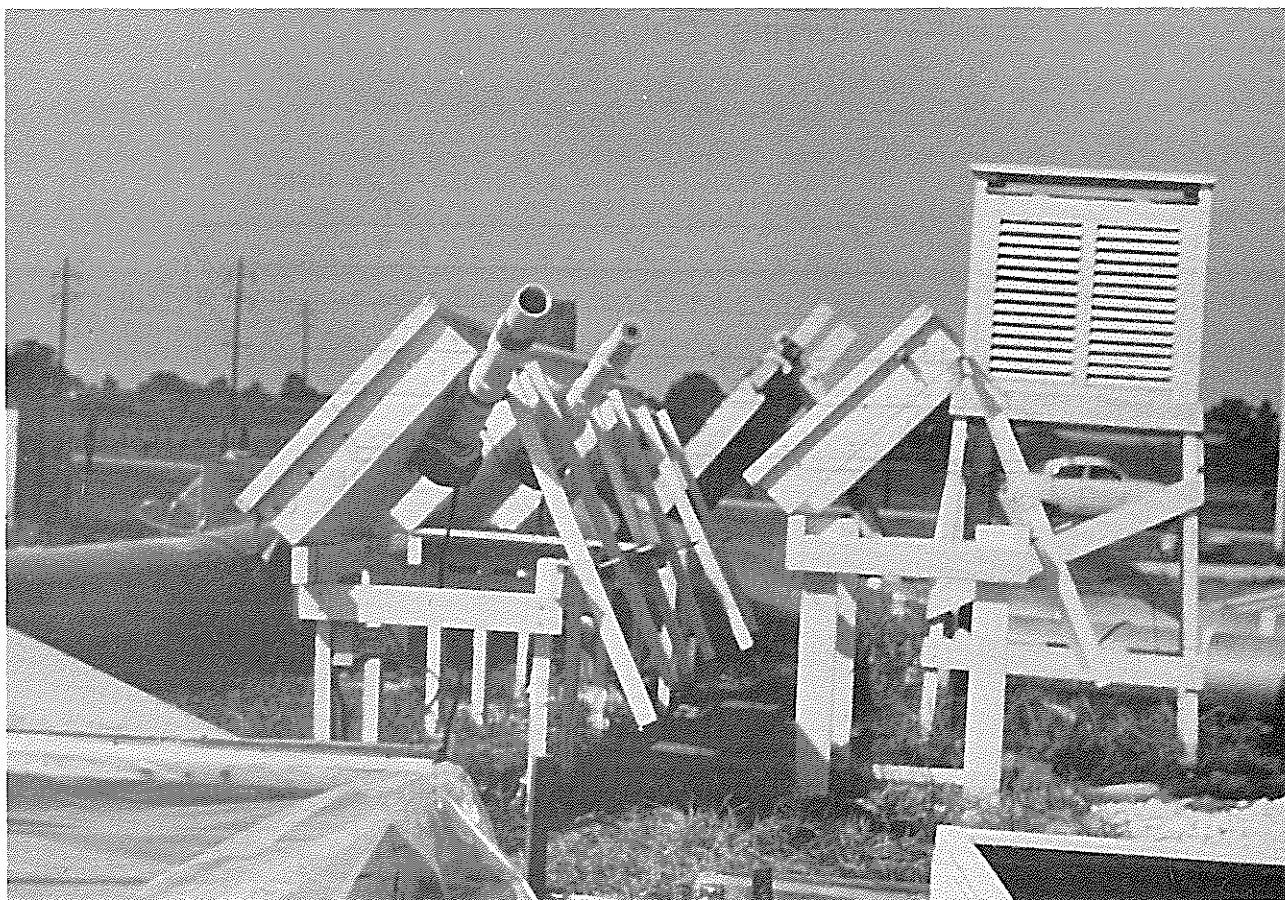


FIGURE 2: Direct Insolation Dryer with Blower

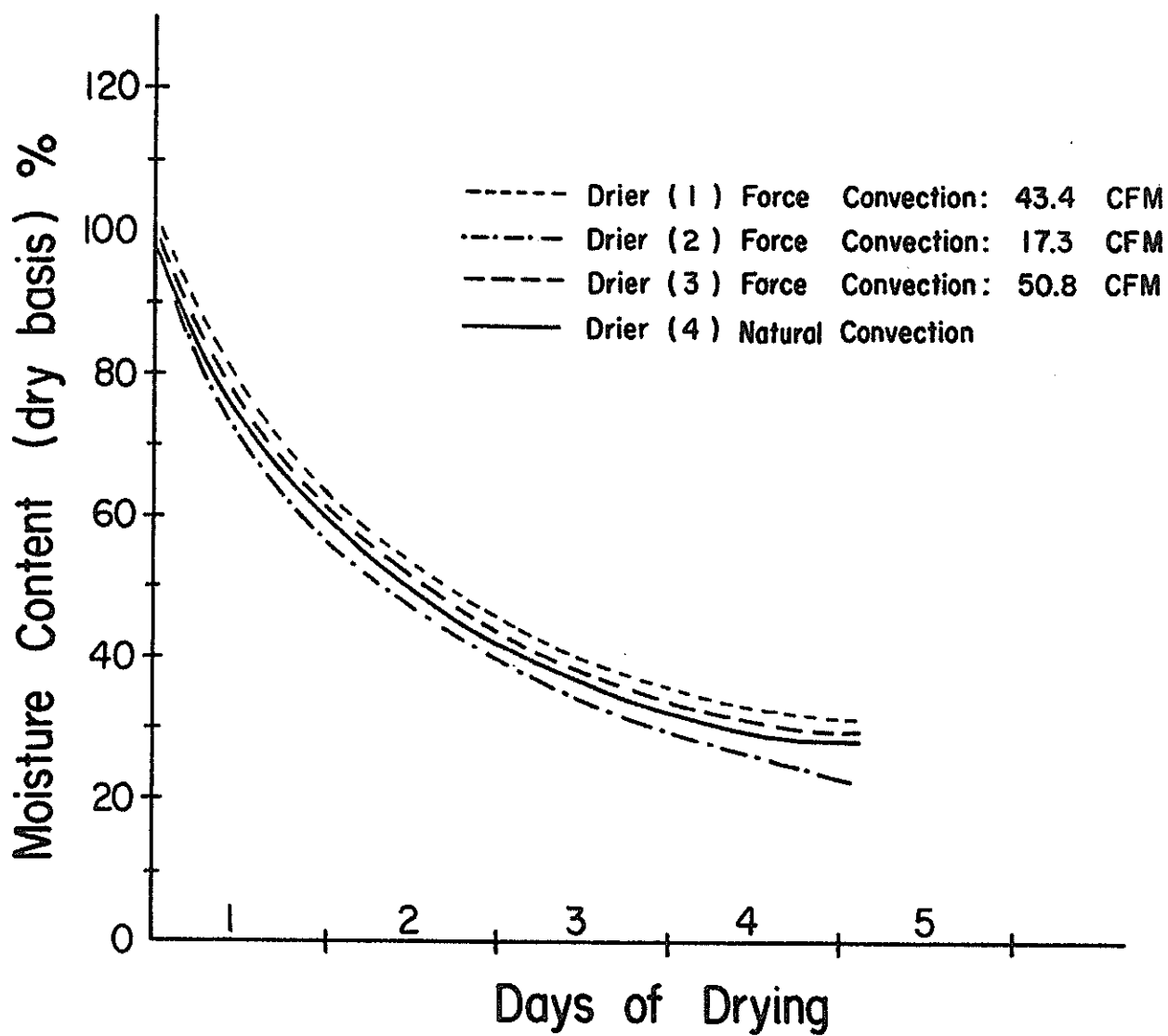


Figure 3. Drying Rate of Mullet Roe in various Air Flow Rate (Sept. 16-20, 1978)

## SOME OBSERVATIONS ON THE BRINING OF MULLET

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It has been estimated that Florida produces some 2 million pounds of smoked mullet per year (5). Of this, the vast majority is produced in small processing plants which have little opportunity for investigation into new or existing products.

Numerous factors are known to affect uptake of salt during brining of fish. Impurities in the salt, fat content of the fish, temperature, agitation, age, species, thickness of the fish and brine concentration have been investigated (3,4,6,8,9). It is generally accepted that during brining, fish gain weight except at very high salt concentrations. An internal brine concentration of 10% appears to be necessary for the shift from weight gain to loss (6). The external brine concentration necessary to reach this level will depend upon a number of factors and is quite high, usually greater than 16%.

Little work has been done on the brining of mullet. Deng (4) studied salt penetration in fresh and frozen mullet fillets and found that salt penetration initially increased with frozen storage, decreased with time and then leveled off following about 5 weeks of storage. Water loss or gain was also observed, with the critical brine concentration being about 20%. No observations were made of the brine concentration necessary for maximum water uptake or its effect on yield following smoking.

Current work in our laboratory has shown that a highly acceptable product is produced by the cold smoking of mullet. A previous investigation (7) demonstrated that yield, chemical stability (rancidity) and sensory acceptance of the product was quite good. However, microbial growth on the product was rapid unless frozen storage was used.

In order to gain additional insight into the manufacture of cold smoked mullet, a study was initiated to observe some of the factors involved in the brining of mullet for this process. Whole mullet were used mainly because of the potential economic advantage of this method of preparation.



## MATERIALS AND METHODS

Mullet (Mugil cephalus) were purchased from various commercial fishermen along the Gulf coast of Florida. They were brought to the laboratory at Gainesville on ice. If not processed within 24 hr, the fish were placed in plastic bags and stored at  $-30^{\circ}\text{F}$ . The day of brining, the fish were thawed in running water, cleaned (gutted and gilled with heads on), and allowed to drain for 30 min. One-half of the fish in each trial had the scales removed. A limited number of studies were also conducted using unscaled butterflied fish.

Brine solutions of 0, 4, 6, 8, 10, and 12% salt (w/w) were prepared the morning of brining and stored at  $40^{\circ}\text{F}$ . The fish were weighed and placed in plastic buckets. Brine solutions in a ratio of 1.5 parts (w/w) brine for one part fish were added to the buckets. Approximately six fish were used per brine treatment with the scaled and unscaled fish brined separately. Each study was replicated at least twice.

The fish were brined overnight for 16 hr at  $40^{\circ}\text{F}$ . They were then removed from the brine, drained 30 min, weighed, and either analyzed for water phase salt (WPS), or cold smoked ( $120^{\circ}\text{F}/5$  hr) using hickory sawdust. Following smoking, the mullet were removed from the smoker, cooled 30 min, and weighed. Samples for WPS were obtained by removing the loin muscle, grinding in a electric meat grinder and placing the ground flesh in Whirl-Pak(R) bags. If the samples were not analyzed immediately, they were frozen until needed.

Percent salt and moisture were run in duplicate according to AOAC methods (1).

## RESULTS AND DISCUSSION

Weight gain following brining of whole gutted and gilled mullet is shown in Figure 1. Maximum weight gain occurred at about 6% brine concentration. There was approximately a 2% greater weight increase with scaled vs. unscaled mullet. If the data for scaled mullet are extrapolated to zero weight gain, the point falls on about 18% brine, very close to the critical range in which mullet fillets either gain or lose weight (4).

This phenomenon of weight gain or loss has been previously reported for fish by a number of workers (3,6,8). Fish muscle protein is altered by salt in such a manner that absorption is increased at low concentrations of salt. At high salt concentrations water is removed by osmotic and other factors. It is interesting to note that our results with the brining of poultry approximate those found with mullet (2). Maximum water uptake, minimum thaw loss and cooking yield data were related to brine concentration. The optimal changes for poultry occurs at about 5% brine.

Water phase salt increased with increasing brine concentration (Fig. 2). Scaling also affected this parameter of the study. Following 16 hr in 12% brine, the scaled mullet had a WPS concentration of

almost 5%, whereas, the unscaled fish contained about 2.5% WPS. When butterflied fish were brined in a similar manner, almost the same trends were observed as with whole mullet. Weight gain was maximal between 4 and 6% brine concentrations and WPS increased linearly (Fig. 3 and 4).

Following cold smoking of the whole mullet, the range of minimum weight loss coincided with that of maximum weight gain during brining (Fig. 5).

Generally, muscle protein solubility is maximum at an ionic strength of 1.1 M, which is close to 6% salt. The maximum yield of cold smoked mullet at 6% brine, indicates that the water is tightly bound by fish muscle protein under these conditions.

Preliminary studies have indicated that taste panel participants prefer a salt concentration for cold smoked mullet within the range that would be considered the most advantageous from a processing standpoint. In comparing 0% to the use of a 6% brine, there would be greater than a 10% increase in overall yield of finished product if optimal conditions were selected.

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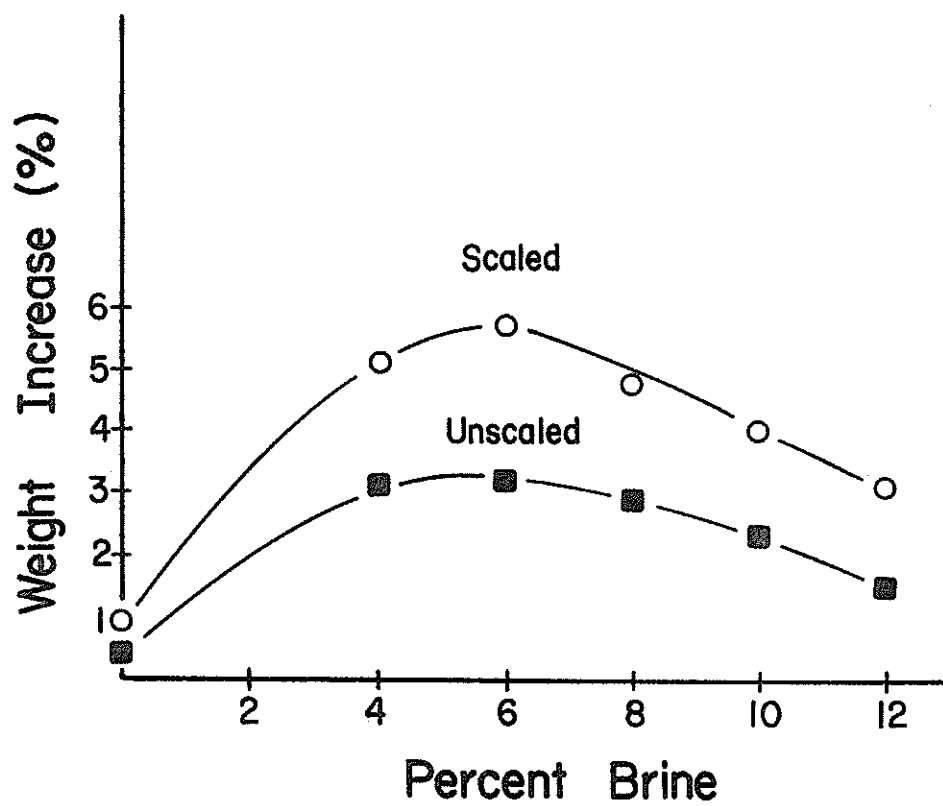


Figure 1. Weight gain following brining of whole gutted and gilled mullet.

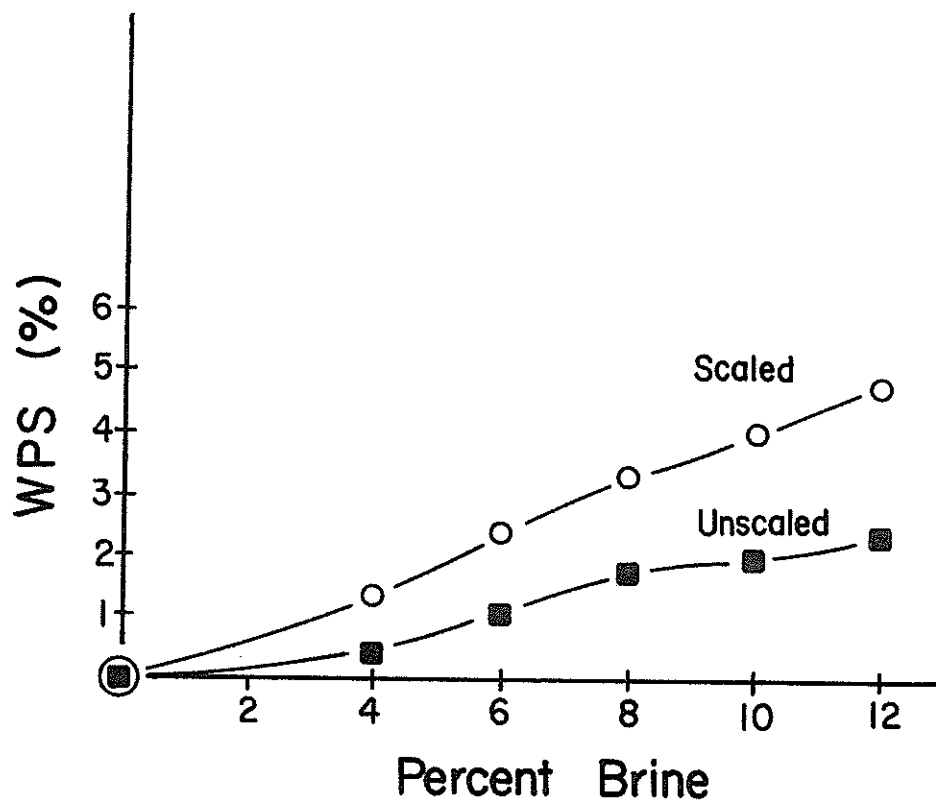


Figure 2. Change in water phase salt of whole gutted and gilled mullet following 16 hr of brining.

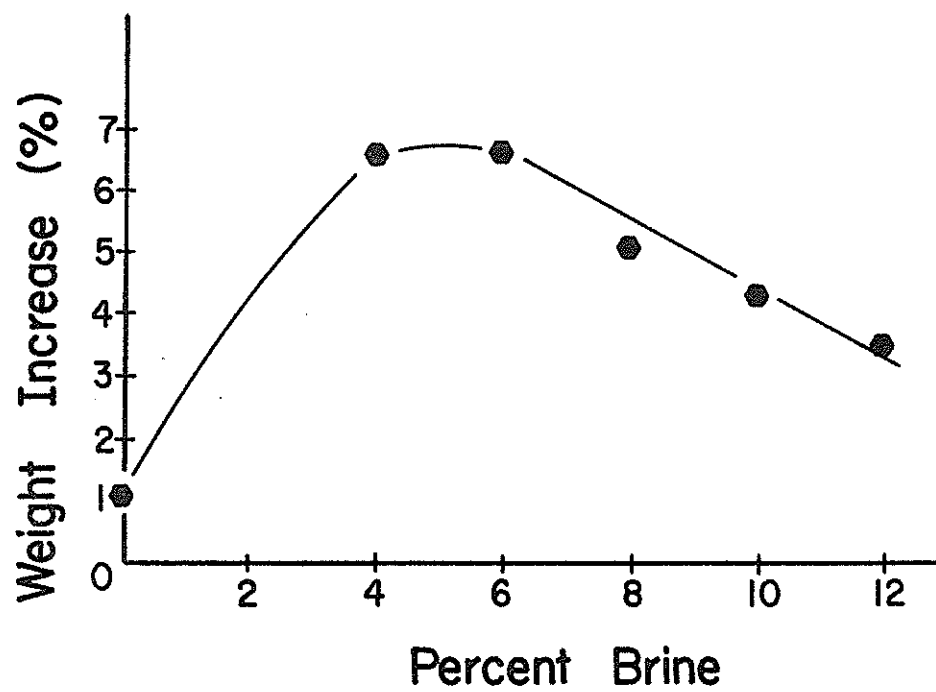


Figure 3. Weight gain of butterflied mullet following brining.

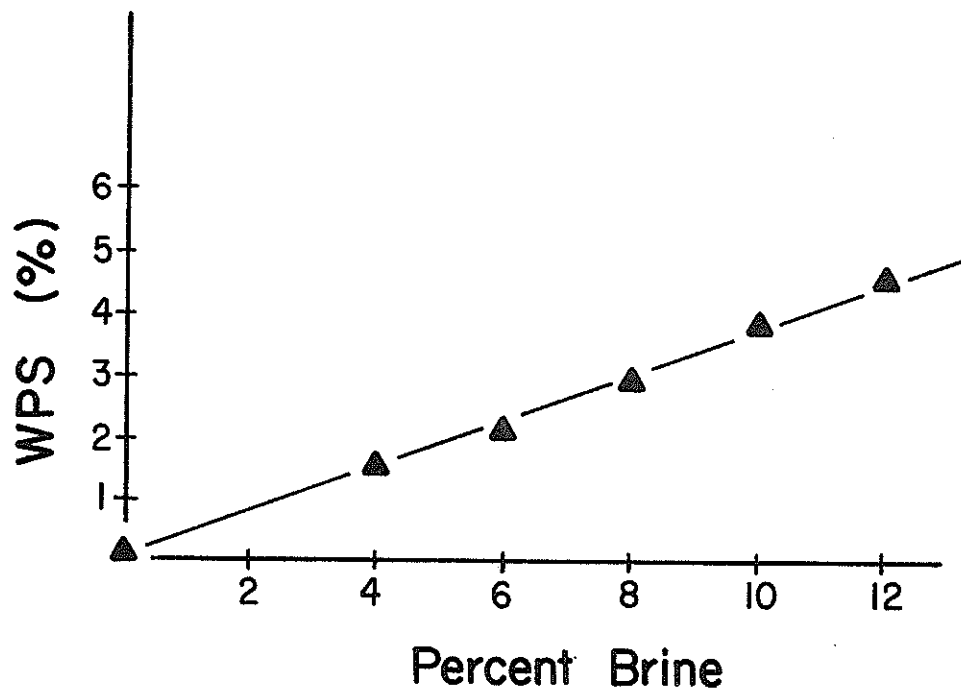


Figure 4. Change in water phase salt of butterflied mullet

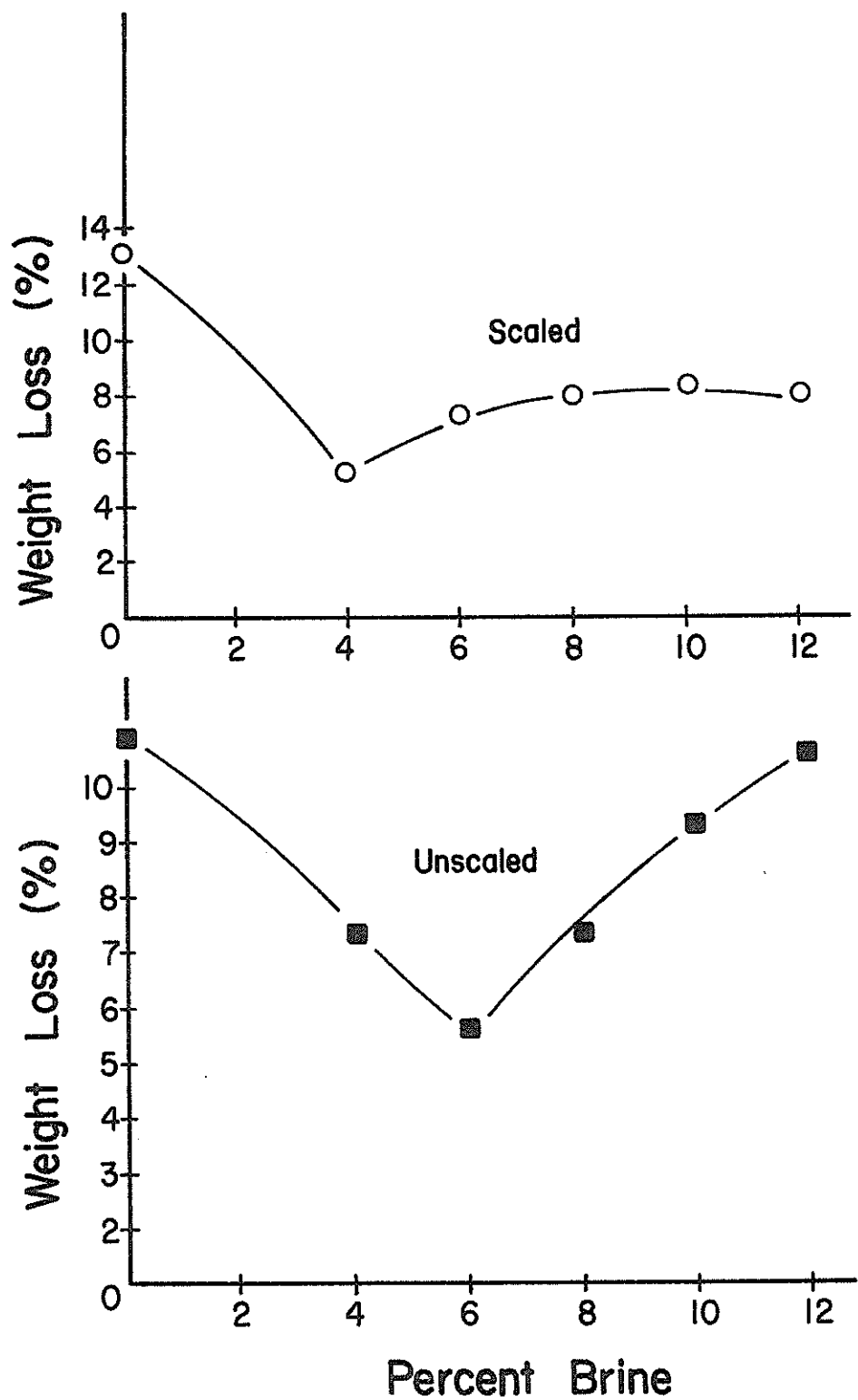


Figure 5. Weight loss of whole gutted and gilled mullet following smoking at 120°F for 5 hr.



EXTENSION OF FLOUNDER SHELF-LIFE BY  
POLY (HEXAMETHYLENEBIGUANIDE HYDROCHLORIDE)

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INTRODUCTION

The high perishability of fresh fish is a serious problem to the seafood industry. While there are many factors (21) involved in the spoilage of fresh fish, under refrigerated conditions, certain microflora such as *Pseudomonas* are believed to play the key role in developing off-odor and other deterioration phenomena (6,7,9,18). Hence, most attempts for preservation of fresh fish have been aimed at inhibiting the growth of these microorganisms (28). The antibiotic chlortetracycline was found to extend the refrigerated shelf-life of fresh fish by several investigators (11,22,27). Such studies led to the approval of chlortetracycline by the U.S. Food and Drug Administration for treating fish (10). However, subsequently, in 1967, this approval was withdrawn as part of FDA's efforts to limit the use of medicinal compounds in foods (24).

Other attempts for prevention of fish spoilage include the use of ethylenediaminetetraacetic acid (5), irradiation (8), combination of irradiation and antibiotic (19), and vacuum or gas flush packaging (1). The old approach of using ice or refrigeration still remains the most practical method of storing fresh fish. Depending on the species this, however, keeps them fresh or acceptable for only 4-6 days beyond the day of capture. Because of this high perishability the marketing and distribution of fresh fish is usually under severe constrain. This leads to higher price of fish or fish products for the ultimate consumers. Hence, there is certainly a need for an effective method to preserve fresh fish.

Poly (hexamethylenebiguanide hydrochloride) or PHMB has been used as a biocide for several years in Australia, Canada, Japan, and in many of the European countries. Under the trade name "Vantocil IB" it has been marketed for industrial disinfection (3) and for short-term preservation of cattlehides and sheepskins (13,14). Under another trade name, "Bacquasil SB," it has been marketed as a swimming pool sanitizer in the United Kingdom (15), Australia, France and Spain (J. Loy, personal communication). In 1975 Strandkov and Bocklemann obtained a U.S. patent (26) for preservation of beverages such as beer by incorporating PHMB up to a level of 50 ppm. Very recently Islam and Islam (16) doubled the

shelf-life of fresh poultry by dipping chicken carcasses in 200 ppm PHMB.

In view of the above applications, PHMB seems to have a good potential for use as a fish preservative. Hence, this study was undertaken to assess the efficacy of PHMB in extending the shelf-life of fresh fish. Flounder was used as a model because of its abundance (23) and importance as a fresh seafood in the U.S. In 1977, Flounder ranked third, behind Tuna and Salmon, in total catch and dollar value (29).

## MATERIALS AND METHODS

### Sample

Summer Flounders, Paralichthys dentatus (Linnaeus), used for this study were caught by a commercial trawler on August 17, 1978 along the Atlantic coast near Ocean City, Maryland. They were caught about 3 miles off-shore at a depth of 10-12 fathoms. One hundred and twenty Flounders weighing between 1-1.5 lb. were eviscerated at the dock facilities of Davis and Lynch Fish Co. (Ocean City, MD), packed in styrofoam containers with plenty of ice (1 part fish to 1 part ice) and then transported to the Food Science laboratory of the University of Delaware at Newark, Delaware.

### PHMB Solutions

Four double layered 30 gallon size plastic bags, placed in rigid containers were each filled with 20 liters of distilled water. "IL-780", a 20% solution of PHMB (obtained from the ICI Americas, Wilmington, Delaware) was added to the water in appropriate amounts so that the final concentrations were 0, 100, 200, and 300 ppm, respectively. The bags were thoroughly shaken, and then stored in a walk-in cold room at  $1 \pm 0.5^\circ\text{C}$  for at least 8 hours prior to use.

### PHMB dip

The eviscerated Flounders in lots of 28 were randomly placed in the four bags and allowed to remain submerged for 2 hours at  $1 \pm 0.5^\circ\text{C}$ . They were then drained for 5 minutes on alcohol swabbed metal racks and individually packaged in gallon size clear polyethylene bags. The packages of each lot were placed in a 30 gallon size dark green plastic bag and stored in an incubator at  $1.1^\circ\text{C}$ .

### Aerobic psychrophile count

Following the 2 hour dip on day 0, and every alternate day thereafter, two fish from each lot or treatment were swabbed for total aerobic psychrophile count. The polyethylene bags containing each fish were placed over ice with the white side (arbitrarily) up. A 9 sq. cm. (3 cm. x 3 cm.) surface area at the widest portion of each fish was swabbed with a sterile aluminum foil template. Appropriate dilutions were made in 0.1% peptone and spread

plated in duplicate on tryptic soy agar (TSA, Difco). The plates were incubated for 10 days at 7°C for enumerations of total aerobic psychrophiles. The average number of colonies from the four plates was divided by 9 and reported as log bacterial count per sq. cm. of Flounder surface.

#### Sensory evaluation

Immediately after the microbial swabbing on day 2, and every alternate day thereafter, the eight Flounders were subjected to sensory evaluation. A six-membered panel consisting of three faculty members and three graduate students evaluated the intensity of off-odor on the fish samples. The following statement was on the evaluation forms, "please open each bag, smell the fish and then rate the off-odor on a 5 point hedonic scale where 1,2,3,4, and 5 represent none, slight, moderate, strong and very strong respectively. A score of 3 or less would indicate that the fish is acceptable to you as a potential consumer". This method is similar to that used by Islam and Islam (16) for assessment of poultry spoilage. When both the fish from the same lot (group) received a score of 5 by at least 4 of the panel members, the remaining fish in that group was eliminated from subsequent sampling and thus no further data were collected on them.

Shelf-life of Flounders in each treatment was calculated based on the number of days the samples from this lot maintained a sensory score of 3 or less. For statistical analysis the shelf-life data were analyzed as a 4 x 6 factorial in a completely randomized design with the treatments and the judges as the main effects. This was followed by Duncan's multiple range test to delineate any statistically significant difference between the treatments (17). To find if there was any overlap between treatments a separate analysis was carried out in a 8 x 6 factorial arrangement with the pair of Flounders and the judges as the main effects.

#### Total lipid and Free fatty acid

On each sampling day, following sensory evaluation, all the eight Flounders were filleted and the total lipid extracted from 50 gram tissue of each fish by the method of Bligh and Dyer (2). For the determination of total lipid an aliquot of 50 ml chloroform extract was concentrated in a rotary evaporator near dryness and then dried for 24 hours at 50°C. The results were expressed as g lipid per 100 g fish tissue.

The free fatty acid (FFA) concentration was determined by using the remaining chloroform extract as outlined by Botta and Shaw (4). The results were reported as g FFA per 100 g total lipid. Statistical analysis was carried

out on the data for each sampling day to determine if the PHMB treatments had any significant effect on the production of free fatty acid.

## RESULTS AND DISCUSSION

### Aerobic psychrophile count

The total aerobic psychrophile counts on the treated Flounders, following PHMB-dip and during the subsequent storage at 1.1°C, are presented in Fig. 1. Two hours immersion in the PHMB solutions resulted in marked decrease in the total number of viable psychrophiles per sq. cm. of the Flounder surface. Following the dip on day 0 the water treated samples on the average had  $3.6 \times 10^2$  organisms/cm<sup>2</sup>. While the 100, 200, and 300 ppm PHMB treated samples had 56, 33 and 22 organisms/cm<sup>2</sup>, respectively. This initial reduction of microbial flora was obviously responsible for the extended freshness of PHMB treated Flounders.

The growth curves for the control group and the 100 ppm group had somewhat similar lag phase which lasted for 2 days following which the exponential phase began for both of them. Whereas the lag phase for the 200 and 300 ppm groups continued till the 4th and 8th day of storage respectively. At the 10th day of storage the water treated controls on the average had a total aerobic psychrophile count of  $15.8 \times 10^6$  organisms/cm<sup>2</sup> while the 100, 200 and 300 ppm PHMB groups had  $4 \times 10^5$ ,  $1.2 \times 10^4$  and  $4.7 \times 10^2$  respectively. Gillespie et al (12) carried out a somewhat similar experiment with eviscerated Sockeye salmon stored in ice containing the antibiotic Chlortetracycline (CTC) at 1 ppm. After 9 days of storage their bacterial counts for the control (ordinary ice) and the experimental samples (CTC & ice) were  $19 \times 10^6$  and  $0 \times 10^6$  per g respectively. After 14 days the CTC - ice samples had  $2.1 \times 10^6$  bacteria per g. Whereas in this study, the 100, 200 and 300 ppm PHMB groups had  $12.5 \times 10^6$ ,  $5.1 \times 10^5$  and  $3.5 \times 10^4$  per sq. cm. respectively.

### Sensory evaluation

The off-odor scores on duplicate samples given by the six judges were averaged and then plotted against the days of storage. These data are presented in Fig. 2. Slight off or fishy odor was detected for all the samples by some of the panel members on the first day of evaluation (2nd day of storage). At the 6th day of storage the water treated samples on the average had moderate (acceptable) off-odor which corresponded to a bacterial count of  $1.2 \times 10^5$  organisms/cm<sup>2</sup> (Fig. 1). By the 10th day of storage the control samples had very strong off-odor while the 200 and 300 ppm PHMB samples had on the average

a slight off-odor. The 100 ppm PHMB samples, however, exhibited moderate to strong off-odor at this stage.

The shelf-life values of all the eight batches (2 batch/treatment) of Flounders are presented in Table 1. As mentioned earlier, these shelf-life data were calculated based on the maximum number of days Flounders in each batch maintained an off-odor score of 3 or less. Analysis of variance on the 8x6 factorial arrangement indicated highly significant ( $p < 0.01$ ) difference in the shelf-life values of the eight batches of Flounders. However, the Duncan's multiple range test exhibited a high degree of uniformity between the batches in each treatment. Except for the 300 ppm PHMB group there were no significant difference between the batches within each group. Whereas the analysis of variance and Duncan's multiple range test on the 4x6 factorial arrangement showed a highly significant difference ( $p < 0.01$ ) among the shelf-life values of the four treatments. Thus it is obvious that the large differences in the shelf-life values of the Flounders were definitely due to the PHMB treatments. The lack of uniformity within the 300 ppm PHMB group may have resulted from a difference in the original bacterial load or the handling during the evisceration prior to PHMB dip.

Overall, the shelf-life improvement by PHMB is highly encouraging. The treatments with 100, 200 and 300 ppm PHMB resulted in shelf-life values of 8.8, 14.0 and 17.2 days, respectively, compared to 6 days for the water treated control. The 17.2 days shelf-life with 300 ppm PHMB indicates that higher shelf-life may be expected with increased amounts of PHMB in the dipping solutions. However, from the standpoint of efficiency 200 ppm treatment appears to be the optimum. This degree of shelf-life improvement is certainly comparable to those obtained by Chlortetracycline (5), irradiation (8), or the gas flush packaging (1).

#### Total Lipid and Free fatty acid

The total lipid content of Flounders used in this study did not change significantly during the storage at 1.1°C regardless of the type of treatment. The lipid content ranged from 0.62% to 0.78% with a grand mean of 0.70%. Thus the lipid content of Summer Flounder is slightly lower than that of Winter Flounder which is 0.8% (25).

Data on percent free fatty acid in the lipid extracted from PHMB treated Flounders are presented in Table 2. The control sample on day 0 of storage had a FFA value of 7.6%. The lowest value (5.3%) for FFA was observed on the 2nd day of storage for 100 ppm PHMB group while the highest value (22.8%) was on the 24th day of storage for the 300 ppm PHMB group. Overall free fatty acid concentration increased significantly ( $p < 0.01$ ) during the storage

regardless of the treatment. However, in general, the FFA values at each sampling day did not differ significantly ( $p > .05$ ) among the four treatments. Thus the PHMB, regardless of concentration, did not affect the production of free fatty acid in the Flounders although there were large differences in the bacterial count. This finding is similar to that observed on antibiotic treated herring (20).

The improvement of Flounder shelf-life by Poly (hexamethylenebiguanide hydrochloride), as observed in this study, is certainly very encouraging. Based on the strong bactericidal action of PHMB on Flounder surface, it is reasonable to expect that the spoilage organisms on other fish and shellfish would be similarly affected. Additional studies on the application of PHMB for extension of Croaker and Oyster shelf-life are currently in progress in this laboratory.

#### ACKNOWLEDGMENT

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Table 1. Shelf-life of eviscerated Flounders at 1.1C following immersion treatment in various solutions of Poly (hexamethylenebiguanide hydrochloride)

Treatment PHMB (ppm)	Shelf-life (days)#	
	Individual batch of Flounders* Mean $\pm$ S.D.	Overall mean for each treat- ment
0	5.8 $\pm$ 0.4 a	6.0 w
	6.2 $\pm$ 0.4 a	
100	8.3 $\pm$ 0.8 b	8.8 x
	9.3 $\pm$ 0.8 b	
200	14.5 $\pm$ 1.8 c	14.0 y
	13.5 $\pm$ 1.1 c	
300	16.5 $\pm$ 0.8 d	17.2 z
	17.8 $\pm$ 1.1 e	

#Means followed by different letters within each column are significantly different ( $p < 0.01$ ) according to Duncan's multiple range test.

\*Standard deviation; six judgements on each Flounder.

Table 2. Percent free fatty acid (FFA)# in the lipid extracted from Poly (hexamethylenebiguanide hydrochloride) treated Flounders during storage at 1.1C

Days of Storage	PHMB treatments			
	0 ppm	100 ppm	200 ppm	300 ppm
0	7.6	7.4	8.0	8.3
2	7.5	5.3	9.7	9.5
4	10.0	8.2	10.3	10.6
6	13.3	10.5	9.6	12.6
8	14.3	13.3	13.6	14.2
10	13.7	15.8	16.1	14.3
12	—*	16.7	15.2	16.4
14	—	15.8	17.9	17.3
16	—	—	18.5	20.1
18	—	—	17.0	20.0
20	—	—	—	21.5
22	—	—	—	20.4
24	—	—	—	22.8

#Based on duplicate samples.

\*FFA values were not determined on Flounders exhibiting very strong spoilage odor.

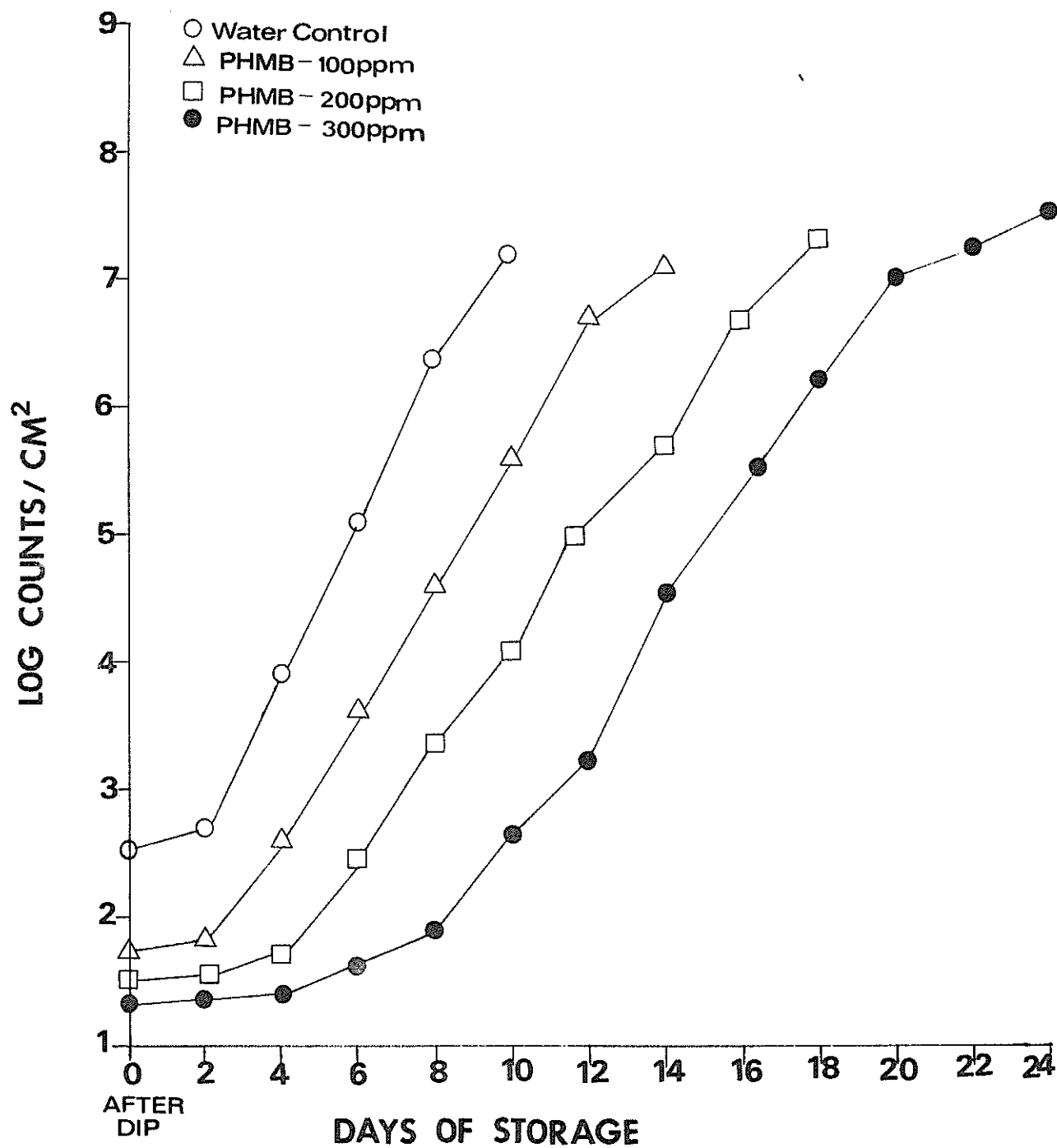


Fig. 1. Total aerobic psychrophile counts of eviscerated Flounders during storage at 1.1C following immersion treatment in various levels of poly (hexamethylenebiguanide hydrochloride).

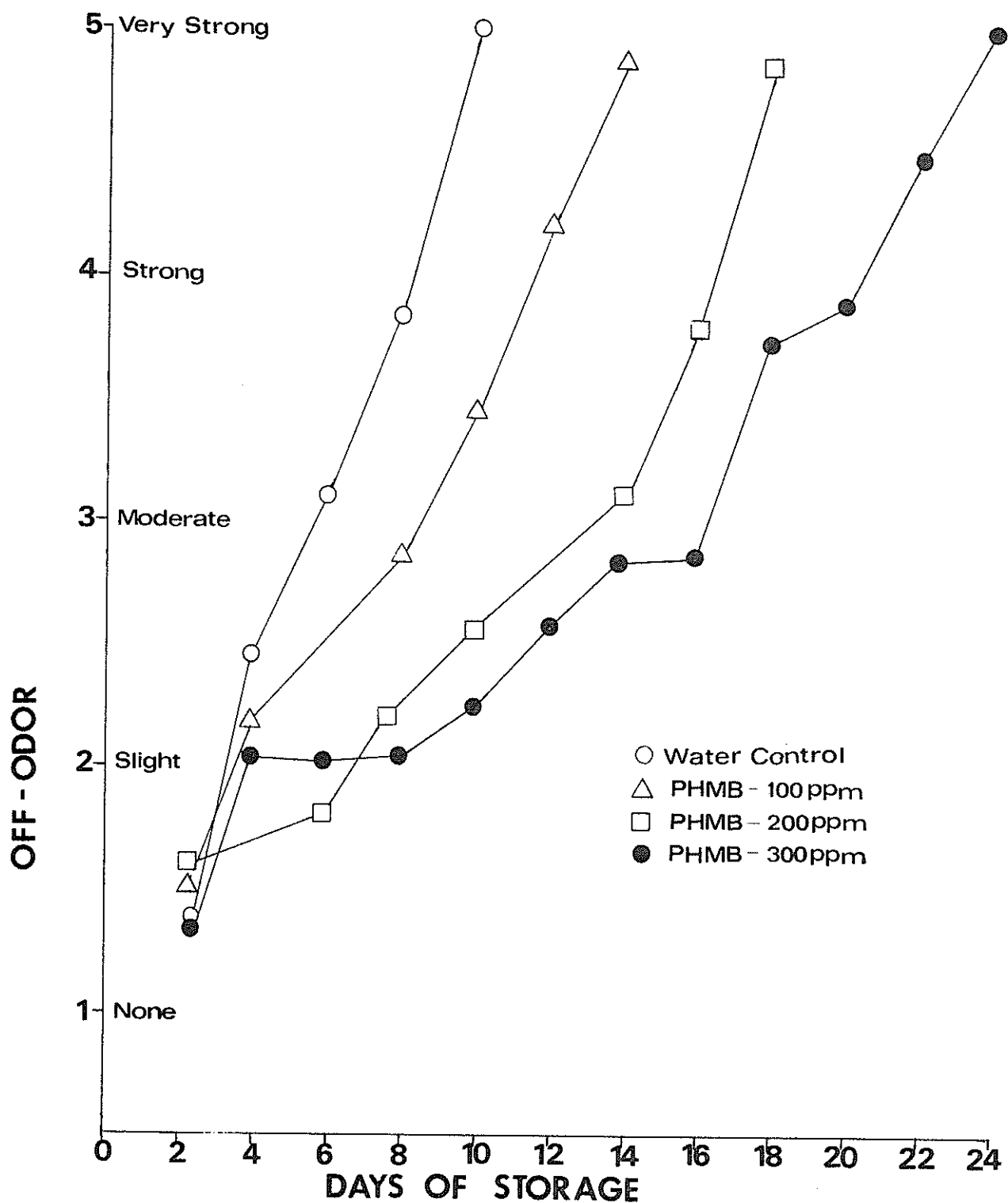


Fig. 2. Off-odor development on eviscerated Flounders during storage at 1.1C following immersion treatment in various levels of Poly (hexamethylenebiguanide hydrochloride).

## FREEZER STORAGE OF BLUE CRABS FOR USE IN PICKING PLANTS

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The seasonal harvest of blue crabs in many states presents significant problems for the industry. During periods of low catch, crab processing plants are forced to close, laying off pickers. During periods of peak catch, an oversupply prevents crabbers from taking all they could harvest because crabs must be processed immediately. The availability of a year-round supply of crabs would utilize this small but necessary labor force of crab pickers to its fullest extent. Likewise, development of a method to preserve crabs for picking at a later time would provide crabbers an opportunity to expand the catch. Partial processing and freezing of crabs during times of abundance for picking when harvest is insufficient, represents one possible solution to the problem.

Ampola and Learson (1) demonstrated that cooked, cleaned crab bodies (cores) could be quick frozen and stored for up to five months with no organoleptic quality loss. However, to our knowledge this procedure is not being practiced in the crab industry.

Discussion with industry personnel provided some insight into information they felt important to know before engaging in such a freezing process. Items included a knowledge of the effect of freezing on (i) the flavor, texture or appearance of the meat [palatability]; (ii) the ease with which the meat is removed from the shell [pickability]; (iii) the lump meat yield and quality; (iv) the total meat yield; (v) the keeping quality of the picked meat; and (vi) the cost involved in freezing and holding the crabs.

This paper presents results of a study designed to address the questions posed by industry personnel about freezing of crabs for later picking. Reported here in detail is information on palatability tests and shelflife storage studies. Pickability and yield studies will be dealt with in general terms. The economics of freezing were not dealt with in this study.

### MATERIALS AND METHODS

#### Crab Processing Methods

Where possible, use was made of commercial facilities for processing, freezing, storing and picking of the crabs.

1. Freezing of Whole Crabs. This processing method was suggested by a local crab processor. Live crabs were precooked in boiling water for three minutes, air cooled and declawed. The whole crabs were quick frozen, placed in boxes, double bagged in 4-mil thick polyethelene bags and stored at -20 C. Frozen crabs were prepared for picking by cooking in boiling water for 12 minutes, air cooling, debacking, cleaning of gills and viscera and washing before placing on picking tables. Crabs processed by this method are referred to as "frozen whole" crabs.

2. Freezing of Cleaned Crabs. This processing method was suggested by Ampole and Learson (1). Live crabs were cooked in boiling water for 10 minutes, removed from the cooker and allowed to cool. Claws were removed from the crabs before debacking, cleaning and washing. The crab cores were quick frozen, placed in boxes, double bagged in 4-mil thick polyethelene bags and stored at -20 C. Crab cores were prepared for the picking tables by dipping in boiling water for three minutes to thaw. Crabs processed by this method are referred to as "frozen cleaned" crabs.

3. Fresh Crabs. This processing method represents the standard procedure used on the Gulf Coast. Live crabs were cooked in boiling water for 15 minutes, air cooled, declawed, debacked, cleaned and washed. Crabs processed by this procedure were used as comparison standards for frozen crabs.

#### Pickability and Yield Measurements

Three pickers were randomly supplied samples of fresh, "frozen whole" and "frozen cleaned" crabs in a blind test. After picking each sample of crab, the pickers were asked to rate the sample on ease of picking and the quality of the lump meat. The weight of the crabs supplied to the pickers and the weight of the flake and lump crabmeat were recorded.

#### Storage Life Test

Samples of meat from crabs processed by each procedure were returned to the lab and placed in ice storage. At regular intervals samples were taken and subjected to the standard 35 C plate count (2) to enumerate the bacteria.

#### Palatability Test

Portions of the meat recovered from the pickability test were supplied to the National Marine Fisheries Service, Technology Laboratory, Pascagoula, Mississippi, for testing by a trained taste panel. Using the triangular test, both chilled and broiled crabmeat samples were rated by the panel on texture, appearance and flavor. An overall rating was assigned to each sample by considering flavor twice as much as appearance and texture and then normalizing to a scale of 100.

## Experimental Conditions

1. Freezer Trial I. Crabs used in this trial were smaller and of poorer quality than the ones preferred by local processors but were used because they were the only ones available at the time. Following processing, the crabs were frozen in a commercial liquid nitrogen spray freezer. The frozen crabs were stored in the freezer of a crab processor. The temperature of the freezer was reported to be -20 C but there is some doubt that the crabs remained at that temperature because they were stored near the freezer door.

2. Freezer Trial II. Crabs used in this trial were classified by a crab processor as being of good size and excellent quality. Because no commercial freezing equipment was available when this trial was started, the crabs were frozen by immersing in liquid nitrogen for two minutes. This procedure caused the shells of the crabs to fracture; a problem which influenced the results of the pickability test. The crabs were stored in a freezer at GCRL where the temperature was verified to remain at -20 C.

## RESULTS AND DISCUSSION

### Freezer Trial I

Following storage in the freezer for 7-1/2 months, the crabs were removed for final processing and picking. The fresh crabs used for comparison were larger in size than the frozen crabs. This fact appears to have biased the pickability test in which a definite preference was shown for the fresh crabs.

Meat yield from the "frozen cleaned" crabs was 15 and 20% higher than from the fresh and "frozen whole" crabs, respectively. However, in another comparison with crabs from this same lot, other pickers were able to recover identical amounts of meat from both the "frozen whole" and "frozen cleaned". Therefore, differences in the first meat yield appear to be related to the consciousness of the pickers rather than a true difference in yields.

Pickers rated the crabs which had been frozen highest in the quality and pickability of the lump meat.

The storage life of crabmeat as assessed by bacteriological counts did not appear to be significantly altered by the freezing process (Table 1). Meat from the "frozen cleaned" crabs had a higher bacterial load than the other meats, a problem which was traced to an improperly sanitized washer used in the pre-freeze portion of the process.

Palatability tests revealed a distinct difference among the crabmeats (Table 2). When chilled crabmeat was served to the trained panel, the meat from the fresh crabs was preferred and that from the "frozen cleaned" ranked second. The difference appeared in all three test categories as well as the overall rating. The differences were less among the crabmeats when served broiled, but they ranked in the same order.



Table 1. Bacteria counts on crabmeat from Freezer Trial I during storage life study.

Days After Picking	Standard Plate Count (35C) per Gram of Meat		
	Fresh	Frozen Whole	Frozen Cleaned
0	65,000	62,000	169,000
2	16,500	67,000	64,000
4	52,000	49,000	99,000
6	60,000	73,000	137,000

Table 2. Results of palatability test\* on flake crabmeat from Freezer Trial I.

Source	Appearance	Flavor	Texture	Overall Rating
<u>Chilled Crabmeat</u>				
Fresh	7.00	6.50	6.75	83.59
Frozen Whole	5.50	4.50	5.13	61.34
Frozen Cleaned	6.88	5.50	6.38	75.81
<u>Broiled Crabmeat</u>				
Fresh	7.00	7.25	6.75	88.28
Frozen Whole	7.13	5.88	6.25	78.56
Frozen Cleaned	7.00	6.25	6.50	81.25

\* Test based on 8-point scale with scores on individual characteristics of less than 5 or on the overall rating of less than 62.5 indicating that there was some degree of dislike of the product.

## Freezer Trial II

In this trial, crabs were held in the freezer for six months before being removed for final processing. As noted in the methods section, the freezing technique which was employed in this trial caused the shells of the crabs to fracture. This was a most significant problem with the "frozen cleaned" crabs. The breaking of the shells as the crabs were being picked caused the pickers to rate the frozen crabs as less desirable to pick. Lump and total meat yields were highest from crabs which had been "frozen whole". Again, however, other factors probably biased the yield results.

The keeping quality of the meat from the frozen crabs was superior to that of the fresh crabs (Table 3) as indicated by a slower rise in the bacterial count.

Palatability tests showed that crabmeat picked from the frozen crabs in this trial was equal to or better in all respects to the meat picked from fresh crabs (Table 4). We feel that two factors, a better quality of crab and a constant low storage temperature, contributed significantly to the improved palatability rating of the frozen crabmeat in the second freezer trial.

### SUMMARY

This paper has presented one possible solution to the problem in the crab industry caused by a seasonal harvest. These studies provide data which indicate that freezing of crabs for storage and later picking is feasible. If crabs are of good quality before freezing and if they are quick frozen and stored under a constant low temperature (i.e. Freezer Trial II) the flavor, appearance, and texture of the crabmeat after final picking and the keeping quality of the crabmeat are excellent. Further, if the crabs are not of good quality and if the freezing and storage processes are not closely controlled (i.e. Freezer Trial I), the processed crabmeat will be of poor quality.

Both methods of processing and freezing crabs gave comparable results. The freezing of cleaned crabs appears to be advantageous in that less weight of material must be frozen and stored, thus reducing cost. However, more care needs to be taken to prevent dehydration of the meat.

Additional research in the areas of yield and economic studies is advised before a commercial application of this process is attempted.

### ACKNOWLEDGMENTS

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Special thanks to the industry personnel who assisted in this project and to the NMFS, Technology Laboratory, Pascagoula, MS, for conducting the palatability tests.

Table 3. Bacteria counts on crabmeat from Freezer Trial II during storage life study.

Days After Picking	Standard Plate Count (35C) per Gram of Meat		
	Fresh	Frozen Whole	Frozen Cleaned
0	7,400	13,000	11,500
4	7,500	8,100	11,000
6	17,000	11,000	15,700
8	120,000	12,000	13,000
11	650,000	10,000	41,000

Table 4. Results of palatability test\* on crabmeat from Freezer Trial II.

Source	Appearance	Flavor	Texture	Overall Rating
<u>Chilled Flake Crabmeat</u>				
Fresh	8.25	8.63	8.75	85.62
Frozen Whole	9.75	9.13	9.00	92.50
Frozen Cleaned	9.63	9.13	9.13	92.50
<u>Chilled Lump Crabmeat</u>				
Fresh	8.88	8.50	8.63	86.25
Frozen Whole	9.75	8.88	9.50	92.50
Frozen Cleaned	9.88	9.25	9.63	95.00
<u>Broiled Flake Crabmeat</u>				
Fresh	8.50	8.88	8.63	87.17
Frozen Whole	9.00	9.13	8.88	90.30
Frozen Cleaned	8.88	8.63	8.88	87.50
<u>Broiled Lump Crabmeat</u>				
Fresh	8.75	8.25	8.75	85.00
Frozen Whole	9.75	9.13	9.38	93.42
Frozen Cleaned	9.75	9.13	9.38	93.42

\* Test based on a 10-point scale with scores of less than 6 on the individual characteristics or less than 60 on the overall rating indicating some degree of dislike of the product.

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## QUALITY AND CHEMICAL STABILITY OF YU-SONE MADE FROM DIFFERENT SPECIES OF UNDERUTILIZED FISH

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Yu-sone, a seasoned and semi-dried fish flake, is a prime candidate for a seafood product developed from underutilized fish species. This traditional Chinese product is a good source of high quality protein, has a relatively stable shelf life, and already has established markets in the Orient. The processing parameters for this product have been studied extensively and the results were reported in last year's meeting (2).

In order to be of interest to domestic processors, various species of finfish with little domestic market must be convertible to an acceptable product. This study was designed to examine characteristics of various fish during and after Yu-sone processing. These species are different from one another in terms of texture, color, fat content, collagen content, and some extraordinary constituents such as urea. Problems were identified for each species and solutions are suggested.

### MATERIALS AND METHODS

#### Materials

Eight species of finfish were used for the Yu-sone produced in this study. Black drum, Gulf sea bass, lizardfish, ray, shark, and triggerfish were supplied by fishing boats owned and operated by the University of Georgia. These fish were caught within twenty miles of the Georgia coast. Fresh croaker and mullet were supplied by a local seafood market. The ray "wings" and shark were filleted, skinned, and frozen at  $-20^{\circ}\text{F}$ . The other species were scaled, dressed, deboned in a Miny Fish Separator (Yanagiya Machinery Works, Ltd., Japan), and frozen at  $-20^{\circ}\text{F}$ . Black drum, ray, shark, and triggerfish were stored for six months and the other species were stored less than one month before being processed.

Two commercial Yu-sone products were used as references. One product was purchased in Taiwan (Brand I) and another from a Chinese food store in New York's Chinatown (Brand II).

Ingredients used in Yu-sone preparation included non-iodized table salt, plain bleached flour, soy sauce, cane sugar, lard, cottonseed salad oil, licorice powder, and traditional Chinese "Five Spice" seasoning.

#### Sample Preparation

Prior to sautéing, the minced frozen fish samples were thawed in cold water, weighed, steam cooked for forty minutes, cooled, and reweighed. The ray and shark fillets were treated similarly, but were boiled in water instead of being steamed.

One mullet sample was prepared by immersing frozen dressed mullet in boiling water for ten seconds. This allows the removal, by hand, of the skin, scales, and outer fat layer (1). After thawing, the mullet was boiled in a 8.74% NaCl solution for ten minutes, hand deboned, and boiled for another ten minutes in fresh water. While deboning, layers of dark flesh were also removed.

Subsequent processing steps were:

- 1) The cooked flesh was weighed and the percentage moisture determined by an Ohaus moisture balance.
- 2) Quantities of ingredients were measured according to the dry weight of the cooked flesh.
- 3) An iron cooking wok was used for sautéing on a thermostatically controlled candy stove as described by Gates and Wu (2).
- 4) When the percentage moisture of the product approached 50%, the cottonseed salad oil was added and mixed with the fish. The wok temperature was then increased to heat up the product to 50°C. For an oil addition study, two modifications to the above procedures were introduced:
  - a) Two mullet and one croaker sample were prepared with lard rather than salad oil.
  - b) Three croaker samples were prepared by adding oil when the product was dried to four different moisture levels; 30, 40, 50, and 60%.

- 5) As the product approached the desired moisture level, "five spice" seasoning was added and heat reduced. Licorice was used for seasoning the mullet sample to neutralize strong flavors.
- 6) After complete dispersion of the spice, the product was allowed to cool and packaged in Whirlpak™ bags.
  - a) Samples for sensory evaluations were stored under refrigeration.
  - b) Samples for chemical tests were kept at room temperature.

#### Objective Analysis

Thiobarbituric acid determinations (TBA) (4) were conducted at three week intervals for a period of twelve weeks. Proximate compositions of products made from the croaker, mullet, ray, triggerfish, and a commercial reference sample (Brand I) were determined for comparative purposes. Analyses included moisture, ash, fat, and protein (3).

#### Sensory Evaluation

Six local Oriental residents participated in taste panel tests on various Yu-sone products which had been stored at 0°C for two and six months. Samples presented to panelists included eight samples, prepared in this laboratory with similar formulations and procedures, and one commercial sample (Brand II).

### RESULTS AND DISCUSSION

#### Oil Addition Study

The products cooked with lard instead of salad oil generally presented a "greasy mouth" feeling and "heavy" oily flavor which was less desirable for the product. To avoid this effect, cottonseed salad oil was used instead for this study.

Noticeable differences in texture, color, and flavor of croaker Yu-sone were observed when oil was added at moisture levels of 30, 40, 50, and 60% during sautéing. When oil was added at the 60% moisture level, it was easier to separate the fibers. The drying speed was also slower which tended to hold the product at a milder sautéing condition and lengthened the cooking time.

When the oil was added at the 30% moisture level, the product had been dried without oil for a period of time which made fiber separation less complete and clumping occurred. At this stage, the added oil did not help to break up the clumps. The final product became dark



and crisp. However, the overall cooking time was shorter.

From these observations, oil addition at 50% product moisture level was chosen as an optimum. This procedure was used in preparing various samples throughout this study.

#### Differences in Characteristics During Sample Preparation

After boiling or steaming, the moisture content of the fish flesh ranged from 71 to 76 percent. With the exception of ray, there was little difference in appearance and texture among different species used. Ray had a long, tough fiber structure that was reminiscent of cooked pork.

During sautéing, many differences became evident in fiber structure, drying rate, and stickiness. Black drum, croaker, Gulf sea bass, lizardfish, and mullet separated very easily into short, loose fibers which sparingly stuck to the wok surface during sautéing. These characteristics resulted in the production of homogenous products. The triggerfish sample did not stick either, but maintained longer fibers throughout sautéing. Shark and ray products stuck excessively to the wok surface and the fibers were hard to break up and mix. This is probably due to the high collagen content in the muscle from shark and ray. With shark Yu-sone, this problem was alleviated with the addition of salad oil. However, some shark fibers clumped into tiny crisp balls which remained throughout sautéing. Ray continued sticking even after oil addition and accumulated on the wok surface as a mass of fiber and oil. With continued sautéing, this fiber-oil mass eventually became part of the product in the form of soft oily clumps which became hard and crisp when cooled.

#### TBA Determinations

All products maintained very low TBA values throughout the three months testing period under room temperature. These values were under 10 or close to zero which is insignificant as shown in the previous study (2) in that rancid odor became detectable when TBA value was near 100. TBA values for products without added oil were approximately 50 for the croaker sample and 120 to 220 for the mullet samples (2). Comparing these results, it indicates that antioxidants present in commercial oil had a great effect on depressing the detectable TBA values in Yu-sone products. The organoleptic test also indicated that rancidity was not detectable in products with added oil. Thus the addition of oil as the antioxidant appears to be a good solution for the fat rancidity problem of certain species.

### Proximate Composition

On a moisture and ash-free basis, the products prepared in this laboratory contained 41 to 57% protein (Table I), which was higher than the commercial samples containing 29 and 38% protein. It was shown in the previous study (2) that palatable products with protein contents as high as 74% could be produced without added oil. Fat content of our products ranged from 21 to 35%, which was comparable to the 29 and 31% values of the two commercial brands. Due to the inherently high fat content of mullet, adjustment of the formulation may be necessary to lower the fat content of the final product.

The sum of the fat and protein percentages for each of our products was higher than those of the commercial samples. This higher, unaccountable fraction in the commercial samples may be related to the amount of added sugar and flour. The higher sugar content was detected in the sensory evaluation which judged the commercial samples as being sweeter than our samples.

Products with added oil were dried to a moisture content as low as two percent and remained palatable. The resulting product had a desirable crispiness not found in the wetter product. The products with no added oil (2) required a moisture content from 10 to 15% in order to retain both palatability and stability. A higher moisture content promoted microbial growth while a drier product possessed a very dry mouth feel.

Ash content on a dry weight basis ranged from 6.77 to 9.26% for all samples analyzed. Salt content was related to the amount of ash present. Thus, the differences in formulation of the various products were shown to be insignificant in terms of amounts of salt added.

### Sensory Evaluation

Among eight products prepared in the pilot plant, no great differences could be measured by panelists on samples stored for either two or six month periods (Table II and III). Yu-sone from shark, ray, lizardfish, and mullet had slightly favorable scores for texture at both two and six months. Both ray and shark were judged higher on flavor and odor after two months storage, but after six months storage; ray, lizardfish, and Gulf sea bass products were preferred. The shark sample became less favorable due to the slight "cardboard box" taste that developed, perhaps due to the urea content in shark muscle. The ray sample had low scores in appearance, probably due to its dark color and dry look. Based on overall comparisons, shark,

ray, and lizardfish were judged as favorable products after two months storage; but ray, lizardfish, and Gulf sea bass were preferred after six months.

The commercial sample (Brand II) scored high at both storage times. This is probably due to both the superior raw material used and extra ingredients added in the product, such as MSG and possibly colorants. It was also judged sweeter than our products which is a preferred, traditional Oriental taste. Since no great objectionable features were detected in most of the samples prepared by this laboratory, they can be improved to the point of total acceptance by adjusting the formulation. Extra handling is needed with shark, such as bleeding and washing to decrease the urea content. Deboned meat seems to require washing before the steaming step so that blood content in the muscle can be reduced.

### CONCLUSION

From this study, we found that palatable Yu-sone products could be produced from various underutilized fish without major problems. These species ranged from ray and shark with long, tough fibers to croaker and lizardfish with short, soft fibers. Adjustments in preparation, formulation, or packaging methods will be needed to improve the quality of certain species.

While increasing quantities of these fish as well as many other species are being landed due to the extended jurisdiction, the Yu-sone industry may be one of the solutions to dissipate these catches. Yu-sone may be exported to the Orient or possibly developed as a domestic item. A mechanized system has been developed in our pilot plant to decrease the labor cost. Its economic feasibility is being studied. The production system will be modified to obtain the optimal efficiency and be adapted to the domestic seafood industry.

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<u>PARAMETER</u>	<u>CROAKER</u>	<u>MULLET</u>	<u>RAY</u>	<u>TRIGGER- FISH</u>	<u>BRAND I<sup>c</sup></u>	<u>BRAND II<sup>d</sup></u>
% Moisture	3.77	1.78	3.33	4.61	9.67	2.17
% Ash <sup>a</sup>	9.26	7.79	8.01	7.80	7.91	6.77
% Fat <sup>b</sup>	28.17	35.50	21.49	24.28	29.36	31.35
% Protein <sup>b</sup>	47.01	41.81	57.38	51.97	38.75	29.44
% Fat & Protein Combined <sup>b</sup>	75.18	77.31	78.87	76.25	68.11	60.79

<sup>a</sup> Moisture free

<sup>b</sup> Ash and moisture free

<sup>c</sup> Commercial brand bought in Taiwan

<sup>d</sup> Commercial brand bought in New York City,  
proximate data as determined by Gates and Wu (2).

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TABLE I - PROXIMATE COMPOSITION OF YU-SONE SAMPLES

<u>SAMPLE</u>	<u>APPEARANCE</u>	<u>ODOR</u>	<u>FLAVOR</u>	<u>TEXTURE</u>
Black Drum	3.50±0.55	3.50±0.84	3.00±1.26	2.83±1.33
Croaker	4.17±0.98	3.83±0.75	3.00±1.26	3.67±1.03
Gulf Sea Bass	3.50±0.55	3.50±0.84	3.67±1.51	3.33±1.51
Lizard- fish	3.83±0.75	2.83±0.41	3.50±0.84	3.67±1.21
Mullet	3.67±0.82	3.50±0.55	3.17±1.17	3.50±0.55
Ray	3.17±1.83	3.67±1.03	4.00±1.26	4.33±1.21
Shark	3.83±0.41	4.17±0.41	3.67±0.52	3.67±1.03
Trigger- fish	4.00±1.26	3.33±1.51	3.67±1.21	3.17±1.47
Brand II <sup>a</sup>	3.67±1.51	4.83±0.41	4.33±0.82	4.17±0.98

<sup>a</sup>Commercial brand bought in New York City

<sup>b</sup>Average of the six panelists, based on a 1 to 5 scale with 5 being most favorable and 1 least favorable.

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TABLE II - SENSORY EVALUATION FOR SAMPLES STORED FOR TWO MONTHS

<u>SAMPLE</u>	<u>APPEARANCE</u>	<u>ODOR</u>	<u>FLAVOR</u>	<u>TEXTURE</u>
Black Drum	2.83±0.69	2.83±0.90	3.00±0.58	2.17±0.90
Croaker	3.33±1.25	3.17±1.34	3.67±0.75	2.33±0.94
Gulf Sea Bass	3.33±0.75	3.50±0.76	4.12±0.69	3.17±1.21
Lizard-fish	3.33±0.47	4.17±0.69	4.00±1.00	3.83±0.69
Mullet	3.00±0.58	3.00±0.82	3.17±0.69	3.17±0.69
Ray	3.08±1.00	4.33±0.47	3.67±0.47	4.17±0.37
Shark	4.00±0.82	3.33±0.75	3.00±1.00	3.50±0.76
Trigger-fish	3.33±1.11	3.17±0.69	3.00±0.82	3.17±1.07
Brand I <sup>a</sup>	3.50±1.26	4.67±0.75	4.67±0.47	4.33±0.75

<sup>a</sup>Commercial brand bought in New York City.

<sup>b</sup>Average of six panelists, based on a 1 to 5 scale with 5 being most favorable and 1 least favorable.

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TABLE III  
SENSORY EVALUATION FOR SAMPLES STORED FOR SIX MONTHS

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## DEBONED FISH FLESH FROM NON-TRADITIONAL GULF OF MEXICO FINFISH SPECIES

### 1. Production Yields and Composition.

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Minced or deboned fish flesh offers great processing possibilities and marketing options which up to a few years ago were not readily available for unconventional fish species. The potential for unconventional finfish from the Gulf of Mexico is best exemplified by the by-catch from shrimp trawling operations. According to Bullis and Carpenter (1968), approximately 591,800 tons of fish were taken and discarded in shrimping operations during 1967. The composition of this catch included croakers, spots, seatrout, black drum, and a number of lesser species of bottom fish.

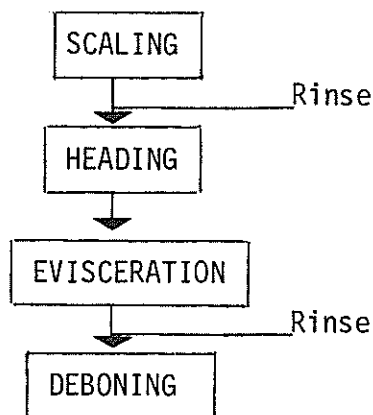
This study was designed to develop information on the yield and composition of minced flesh that can be produced from non-traditional Gulf of Mexico finfish species. In addition to five marine, one fresh water fish which is readily available in South East Texas was also investigated.

### MATERIALS AND METHODS

The fish used in this study were non-traditional seafood species which are readily available on the wholesale market along the Texas coast. Six different species were investigated including: sheepshead, mullet, croaker, sandtrout, black drum and tilapia.

At the time of arrival at the pilot processing plant in Corpus Christi, the 400 pounds were equally divided into two parts; one part was processed immediately while the others were kept frozen in the round and kept in frozen storage at -20° for two weeks. Both the fresh and pre-frozen fish, after thawing, were processed according to the following scheme:





Yield after each processing step was determined starting out with approximately 200 pounds. Total nitrogen of the deboned flesh was determined according to Kjeldahl (AOAC, 1975). Non-protein nitrogen (NPN) was analyzed by grinding the minced fish flesh with a 7% trichloroacetic acid (TCA) solution for 5 minutes. After filtration, total nitrogen of filtrate (NPN) was again determined according to Kjeldahl (AOAC, 1975). Moisture and ash were determined according to AOAC (1975). Total lipids were determined according to the method of Bligh and Dyer (1959).

## RESULTS AND DISCUSSION

The yields during processing are listed in Table 1. These data indicate some of the physical properties that are characteristic for many of the non-traditional finfish from the Gulf of Mexico. These fish in most cases have a large head structure, heavy large viscera together with heavy bone structure with thick skin. The result of processing is thus a low yield regardless of processing method.

The proximate composition of minced flesh from the six species are shown in Table 2. The composition falls within what would be expected values for such products (Jacquot, 1961). Of the fish used in this study, black drum, mullet and croaker had seemingly just completed their spawning cycle, at which point fish are known to be of poor quality with maximum water content and minimal protein and lipids.

Even though yields of deboned fish flesh from Gulf species are somewhat low, the proximate composition indicate that such deboned flesh should be well suited for a variety of fisheries products like; patties, sticks, portions, sausage like products, etc.

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Table 1  
Yields after stepwise processing of  
Gulf of Mexico Finfish

Process	Sheepshead		1) Black drum		2) Croaker		3) Sandtrout		Mullet		4) Tilapia	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
After Scaling	95.8%	97.1	1) —	1) —	94.0	2) —	97.2	95.8	97.	95.2	96.8	95.6
After Heading	60.6%	64.4	72.1	64.6	66.5	—	77.0	77.0	72.0	66.1	68.4	66.2
After Eviscerating	54.2%	55.7	61.4	56.6	56.4	—	71.0	71.0	54.0	46.1	—	—
Finished Product	24.7%	27.5	26.4	22.0	31.3	—	41.7	43.5	24.6	20.0	26.1	24.6

- 1) Black drum was too large for pilot plant scaling.
- 2) Croaker was processed only as fresh.
- 3) Sandtrout was received partially gutted. Evisceration consisted of removal of air bladder and remaining viscera.
- 4) Tilapia was received already gutted.

Table 2  
Proximate composition of minced flesh (weight %)\*

Analysis	Sheepshead		Black drum		Croaker		Sandtrout		Mullet		Tilapia	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
Moisture	79.62	78.62	77.12	80.25	75.22	1) —	78.05	78.25	80.56	81.96	81.80	80.80
Total N	2.34	2.34	2.58	2.63	2.78	—	2.99	2.80	2.27	2.44	2.37	2.51
NPN/100g	0.29	0.29	0.29	0.30	0.27	—	0.27	0.26	0.25	0.27	0.24	0.25
Protein	14.65	14.63	16.13	16.44	17.38	—	18.69	17.50	14.19	15.25	14.81	15.69
Fat	3.21	3.20	2.86	2.22	5.83	—	2.65	2.00	1.89	1.33	2.50	3.40
Ash	1.15	0.84	0.92	0.90	0.71	—	1.03	0.95	0.84	0.84	0.75	0.79

\* Average of three analyses

1) Croaker was processed only as fresh.

DEBONED FISH FLESH FROM NON-TRADITIONAL  
GULF OF MEXICO FINFISH SPECIES

2. Microbiology

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The employment of meat-bone separators (deboners and/or mincers) in the seafood processing industries is directed towards better utilization of existing products and the development of usable products from non-traditional species. The latter is of primary concern in the Gulf of Mexico because of the large shrimp by-catch. Estimates range from 5-20 pounds of fish caught to every pound of shrimp. For the most part, these fish cannot be processed in a conventional manner because of their small size and odd shape. The application of deboners for such fish offers a chance at expanded utilization of this tremendous resource.

Recent literature in the area of minced fish flesh quality (3,4) have reported on the colder-water species such as Cod, Pollock, Whiting, White Sucker, Haddock, Hake, Ling Cod and Ocean Catfish. Both groups of researchers showed that the product was safe and met the standards recommended by the International Commission on Microbiological Specifications for Foods (1). In one of the studies, it was noted that the quality of the finished product was influenced by the condition and operation of the plant. In a plant operating under good conditions the aerobic plate counts (APC) were reported at 67,000/g; whereas, in a plant operating under poor conditions, the APC of the finished product was 1,500,000/g. Another observation was that the minced product usually had a 10-fold higher count than did the starting product. The increases could have been due to the process, growth, and/or the fact that raw material was sampled by rinsing; whereas, the finished was sampled by blending.

The objective of this study was to determine the microbiological profiles of several warm-water species of fish as influenced by minced flesh processing.

MATERIALS AND METHODS

Black Drum (Pogonias cromis), Sheepshead (Archosargus probatocephalus), Tilapia (Tilapia mossambica), Croaker (Micropogon undulatus), Sand Trout (Cynoscion arenarius), and Mullet (Mugil cephalus) were obtained from commercial sources. The selection of these species was based on abundance and price

at the time of processing. They were processed according to the scheme provided in the previous article (1). Samples of product were taken of the whole fish, after scaling, after heading, after eviscerating, after mincing and of the minced flesh after 1 mo storage at -20°F. The sampling procedure utilized varies somewhat from others reported in the literature. At each step, the entire product or remaining product was blended in a Waring blender of commercial size. Appropriate dilutions were spread plated onto standard methods agar and enumerated after 48 hrs incubation at 25°C. The finished product (fresh) was also evaluated at 35°C.

## RESULTS AND DISCUSSION

The ranges in APC as influenced by the process can be seen in Figure 1. It should be pointed out that the initial counts are rather high. This is not unusual considering the fishery for each. They are harvested in small vessels, many without ice, and could be from 4-5 days before reaching the processing plant. Although some improvement in handling can be made, these fish represent what would be available commercially for a minced fish flesh operation. In examining the various processing steps, there is a slight reduction in counts after scaling (S) and heading (H) with increases at evisceration (E) and mincing (M) to the point that the finished minced product (M) has counts similar to the whole fish (W) used in the process. The Croaker (CR) is the only species showing a reduction at evisceration. The fact that the eviscerating equipment was designed for fish like croaker probably explains this. The extra point below Mullet (MU) at evisceration is the result of an extra tap water rinse before mincing. All counts exhibited a 1 to 2-log decrease after frozen storage (FM).

It would seem logical that decreases in counts might occur at each processing step since the scales, head and viscera are reported to contain the highest number of bacteria per gram of material. An examination of counts obtained from the Black Drum (triangle with broken lines on Figure 1) helps explain this unexpected phenomenon. The results are calculated on fish that were sampled in a reverse manner. That is the counts were conducted on the scales, head, viscera and remains and not on the product with these removed. Counts and weights (Table 1) were then used to reconstruct the fish and develop the curve shown in Figure 1. The fact that the remainder accounted for about

TABLE 1. Percentage of weight and bacteria represented by scales, head, viscera and remains from Black Drum.

Portion	% Weight	% Bacteria
Scales	5.5	22.7
Head	35.5	21.3
Viscera	8.8	0.5
Remains	50.0	56.7
Whole Fish*	101.2	99.8

\*Based on totals of other parts

50% of the original number of bacteria explains why the APC for minced flesh is the same as the whole fish on a gram to gram basis.

When compared to the ICMSF recommendations for fresh and frozen fish, the fresh minced product would not comply; whereas, the frozen product would. It is realistic to say that the finished product would meet these standards because the most practical means of processing and storage would be a final frozen block. The counts could be further reduced by instituting better controls from harvest through processing. The ICMSF recommendations for APC is the examination of 5 subsamples, 3 of which can be between  $10^6$  and  $10^7$ /g, but more can be over  $10^7$ /g. They also suggest that the APC be conducted at 25°C. The reason for this lower incubation is evident in Table 2 where a comparison of APC on minced flesh is made at 25°C and 35°C.

TABLE 2. Aerobic plate counts of minced flesh incubated at 25°C and 35°C.

SPECIES	25°C	35°C
Sheepshead	$1.4 \times 10^5$	$< 3.0 \times 10^4$
Mullet	$4.2 \times 10^6$	$7.0 \times 10^4$
Black Drum	$1.9 \times 10^8$	$1.3 \times 10^6$
Sand Trout	$1.4 \times 10^7$	$2.4 \times 10^5$
Croaker	$8.9 \times 10^7$	$2.5 \times 10^5$
Tilapia	$2.5 \times 10^8$	$6.0 \times 10^6$

In all cases the counts at 25°C are 2-logs higher than the counts at 35°C. Licciardillo and Hill (1978) suggests that the higher incubation temperature would indicate processing sanitation; whereas, the lower temperature would indicate fish quality.

#### CONCLUSIONS

Several observations can be made regarding the microbiology of deboned fish flesh from non-traditional Gulf of Mexico finfish species.

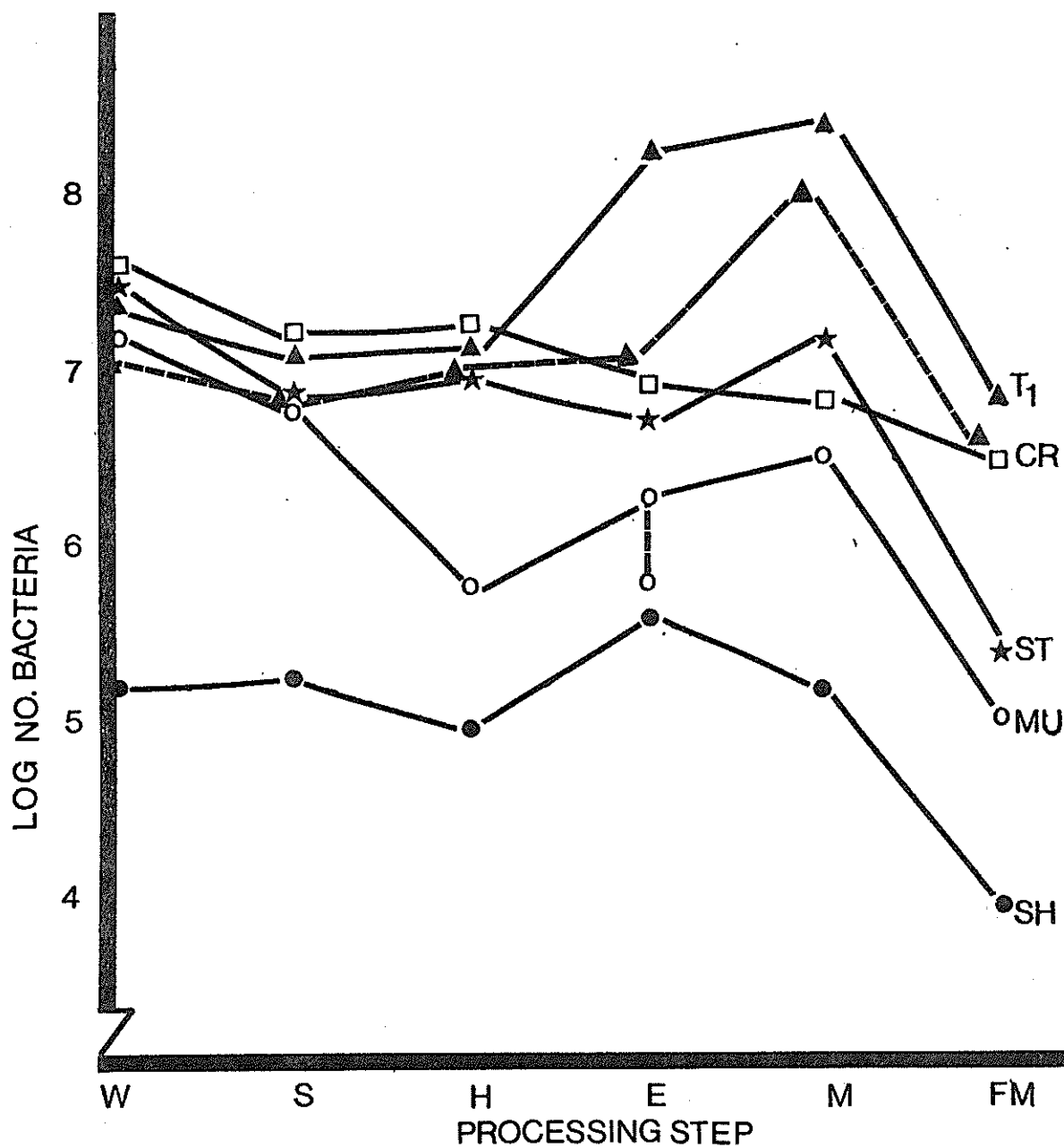
1. The numbers of bacteria in the finished product is influenced by the numbers on the raw whole fish.
2. Freezing reduced the total counts by 1-2 logs.
3. APC was much higher at 25°C than at 35°C.
4. Better quality product can be produced by instituting better controls from harvesting through processing.
5. A comparison of data from various researchers should be examined carefully because of the differences in sampling procedures (rinsing vs blending).

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FIGURE 1. Changes in the number of bacteria per gram of product as influenced by processing.



PRESENT THINKING IN WASHINGTON  
ABOUT  
FISHERIES DEVELOPMENT

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INTRODUCTION

The U.S. fishing industry represents an important segment of the U.S. economy nationally, regionally, and locally. The coastal States contribute directly to fishing and others contribute indirectly in the marketing and distribution of seafood products. The food and industrial goods produced by the U.S. fishing industry are valued at about \$7 billion at the retail level. The industry also creates direct employment and means for more than 250,000 individuals, and produces an important source of food for U.S. consumers--but the opportunities for improvement are large. We have 10 to 20 percent of the world's fishery resources yet we still import two-thirds of the fish we eat. If we put our minds and our abilities to the task, we could double or triple our harvests in the next decade or two. This would have a major beneficial impact on our economy in terms of jobs, GNP and trade deficits.

The Federal role in assisting in development of the U.S. fishing industry has been a subject of increasing discussion in the Legislative and Executive Branches of the Government over the past decade. Congressional actions have been initiated and studies have been conducted on the U.S. fishing industry, but a definitive Federal policy for growth of the U.S. fishing industry has not yet been adopted by the Executive Branch of the Government.

The purpose of this paper is to review Congressional and Executive Branch efforts to arrive at a coherent policy and program in fisheries development.

What is fisheries development? Fisheries development has been defined in different ways by many different people. As we presently use the term in Washington, it refers to two types of activities. The first is in reference to those activities that can be taken to develop a new fishery for the U.S. This includes those fisheries that are predominately harvested by foreign nations as well as those fisheries that aren't being used by anyone. The second category of fisheries development refers to those activities taken to provide supporting services to the existing fisheries so that they can increase their contribution to the national

economy. Thus, there are two types of activities, developing and strengthening, that I will be referring to when I use the term 'fisheries development'.

#### CONGRESSIONAL ACTIONS

I will first provide some background in what Congress has done relative to fisheries development.

Congress has passed many laws which provide direct authorization for Government activities in this area. Among the more important ones are the Saltonstall-Kennedy Act of 1954, the Fish and Wildlife Act of 1956, the Agricultural Marketing Act, and of course, the Fishery Conservation and Management Act of 1976 which established a 200-mile fisheries zone in which United States' citizens have priority access to the fisheries resources (except for certain highly-migratory species). In addition to these laws, there are many others that are directly or indirectly supportive of fisheries development. These laws have provided various agencies with authorizations for Programs in the fisheries development area. These laws have led to activities of the:

- U.S. Army Corps of Engineers, in port and harbor construction;
- the Maritime Administration, in its financial assistance and coastal development planning activities;
- the Small Business Administration, with its financial assistance programs;
- the Economic Development Administration with its technical assistance programs and its ports, harbors, and facilities development programs;
- and on and on.

Congress has also passed resolutions calling for increased recognition of the role of fisheries in the U.S. economy. The most important of these resolutions are the Eastland Resolution of 1973 calling for a strengthening of the fishing industry, and the Senate resolution of March 1979 calling for the Executive Branch to release funds available for fisheries development under the Saltonstall-Kennedy Act.

Congress has also commissioned several studies in recent years dealing with fisheries development. The General Accounting Office study of 1976 dealt with fisheries development in a broad perspective and called for many actions to improve the health of the fishing industry. The Office of Technology Assessment completed a study in 1977 which dealt with the opportunities for expanding and revitalizing the U.S. fishing industry as a result of the 200-mile fisheries jurisdiction as well as matters related to regulation enforcement, management, and information needed for implementation of the Act. The Eastland Survey, completed in 1977, was the most thorough of the reviews. It used a series of nationwide meetings to gather information on the present needs of the fisheries industry. This survey made broad recommendations concerning fisheries management, financial assistance, insurance, tariffs, safety, port and harbor development, charter boats, navigation, marine weather forecasting, fuel

allocation, foreign investment in fisheries, vessel construction, Sea Grant activities, processing, marketing, and aquaculture. The recommendations from this report, while not readily seen as having been implemented by the Government, have been influential in shaping draft legislation and the policy and program being developed by the Fisheries Development Task Force.

There are three bills presently pending in Congress. Two of these, H.P. 2330 and H.R. 2331, have been introduced by Congressman AuCoin. These bills would provide for loan guarantees and tax deferrals for shore-based fish processing facilities. Another bill has been introduced by Senator Weicker. This bill, S. 543, is a very broad bill that contains:

- loan guarantees and tax deferrals for shore-based facilities;
- high-risk loans for fisheries development;
- a national fisheries development board to advise Government on what should be pursued in fisheries development;
- a call for a broad fisheries development program and a national plan for that program;
- a study of the need for training of personnel for fisheries;
- a marketing study;
- a seafood sanitation study and development of a sanitation plan;
- a program to provide broad services to fisheries cooperatives; and
- amendment of the Saltonstall-Kennedy Act to set aside 75 percent of all fisheries import tariffs plus foreign fishing fees and fines into a fund for fisheries development (the present S-K Act sets aside 30 percent of the tariffs on imported fisheries products for fisheries development and other fisheries research).

Other bills are being drafted by industry groups and Congressional committees. These bills contain sub-sets and variations of the elements contained in Senator Weicker's bill. The likelihood of any of these bills getting through the Congressional hurdles is not clear at this time.

#### EXECUTIVE BRANCH ACTIONS

As noted earlier, there are many agencies involved in fisheries development. Just in the Department of Commerce, we find that we have the Economic Development Administration, the Regional Commissions, the Representatives of the Secretary in each region, the Office of Minority Business Enterprise, the Industry and Trade Administration, the Maritime Administration, the Office of Science and Technology, and, of course, the National Oceanic and Atmospheric Administration, or NOAA. Within NOAA we have the Office of Coastal Zone Management, the Office of Sea Grant, and the National Marine Fisheries Service, all having programs that benefit fisheries development.

Last year, NOAA, FDA and four Regional Commissions jointly funded a comprehensive study of domestic and export markets and the impediments to development. With the results of this study starting to come in last fall,

considerable interest was generated in forming a cohesive policy in fisheries development in order to take better advantage of the identified opportunities and to work on reducing the impediments.

A task force on fisheries development was established in late November of last year to sort out the policy and program that the Administration should pursue in fisheries development. It is chaired by Fred Schenck, Deputy Under Secretary of Commerce, and James P. Walsh, Deputy Administrator of NOAA. Joe Slavin and Brian Rothschild, who many of you know, have been in charge of the overall operations of the task force, and I have been the project manager. We have had over 50 staff members in the Department of Commerce plus two contractors working with us to develop the more than 20 background papers and analyses that were used as source documents for the draft policy and program.

I am now going to talk about some of the elements in the draft report of the task force. We are using this draft report to focus discussion on the various needs of industry for assistance in fisheries development, the proper role of Government in rendering that assistance, and various programmatic options that the Government might pursue. Based on the background analyses prepared for the task force and on other studies, we developed a classification scheme for the impediments to fisheries development. The impediments identified and discussed are:

A. The organizational character of the U.S. fishing industry with its many small operating units makes it difficult for the industry to conduct comprehensive planning, develop access to large amounts of capital for new projects, develop new technology or evaluate existing foreign technology, undertake market research, educate consumers about new products, and to organize all the industry sectors required to take a new product from the ocean to the marketplace.

B. We found that infrastructure, or rather the lack of it, is a major impediment to the development of every non-traditional species off the U.S. coast. We need better port and harbor facilities virtually everywhere.

C. We found that there is a need for improved access to capital in some areas. For example, it will take perhaps as much as \$2.5 billion in private capital to develop the Alaska groundfish resources.

D. We found that technology development and technology transfer will be required for the development of many of the new fisheries. While much of the technology required for harvesting and processing is available in the world marketplace, some of it needs adapting to the American fisheries or demonstration of its capabilities to the U.S. industry.

E. Adequately skilled labor is not available in the harvesting or processing sectors to utilize the new technology or techniques that may be required in many of the new fisheries.

F. Government regulations was identified as an area that could inhibit the growth in fisheries. This includes both the fisheries management kinds of regulations which could retard growth if they do not consider the development opportunities, and it also includes the

regulations of other Federal agencies which increase costs for the harvesting and processing sectors. Examples are Environmental Protection Agency effluent guidelines for processing plants, safety and health regulations, and Food and Drug regulations. These regulations need to be coordinated by the Government agencies involved so that the intent of the regulations can be met at the least cost to the industry.

G. We found that another type of impediment lay in the tariff and quota barriers to foreign markets erected by other nations, as well our industry's lack of knowledge of the overseas markets.

H. We found that programs to market fish as well as to win the consumer's acceptance of fish was an area that could be improved. While most people are familiar with the traditional fish species such as shrimp, tuna, and cod, few are aware of the non-traditional species such as mullet, whiting, and squid. There is also a lack of consumer understanding on how to handle and prepare unfamiliar species of fish, consumer concern over product quality, rising prices, and safety of fishery products.

I. Lastly, the existing local state and Federal programs dealing with the impediments that I just talked about are scattered among agencies across the nation and there is no really effective coordinating mechanism. This causes Government difficulty in directing its resources in a coherent program and puts a heavy burden on the industry to coordinate with the many different agencies.

Government has a role in each of these areas under certain conditions. Based on guidance that we have received from upper levels in the Administration and in reading of the draft policy and program of the task force, it is reasonable to state that Government has a role when:

- the relevant industry is too small or fragmented to do the work itself;
- the benefits to the nation far outweigh the costs; and
- Government assistance is not in the form of a subsidy which industry will come to rely on.

There are many programmatic options which lie within this policy framework. Among the many options identified in the draft task force report are:

1. fostering the development of industry cooperatives and development foundations;
2. establishing a national council on fisheries development to advise the Government;
3. establishing national and regional conferences on fisheries development;
4. planning for the infrastructure needs and providing additional resources for their development;
5. extending present vessel loan guarantees and tax deferral

programs to shoreside processing facilities;

6. conducting technology development and technology transfer programs;

7. providing programs to train workers;

8. establishing mechanisms within the Government to coordinate regulations;

9. increasing the recognition of fisheries needs in international trade negotiations;

10. developing better information on overseas markets and conducting trade missions and trade fairs;

11. educating consumers as to the availability and the proper preparation techniques of the new species as well as to their safety and nutrition;

12. conducting research to improve product quality and product utilization; and

13. conducting market research to find new markets for fisheries products.

The general precept being established is a strong requirement for a thorough systems approach to fisheries development. Each fishery or group of fisheries is different in its impediments, the costs to remove them and in the benefits to be derived. Coordinated Government/industry plans will have to be developed before significant Government resources will be released.

What are the next steps?

We are just completing a review of the draft policy and program with industry representatives. We now need to finalize the policy and gain concurrence on it among the Government agencies that impact on fisheries development, and also to determine which of the many program options will be pursued and at what level of funding. We expect to have this all done in time to present a comprehensive program at the National Conference on Fisheries Development on May 23 and 24, 1979, in Washington, D.C.

SEAFOODS - WHAT ADDITIONAL INFORMATION IS NEEDED TO FULLY  
APPRECIATE THIS NUTRITIVE VALUE?

BY

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Seafoods should play a more important role in the American diet. To ascertain this role more scientific information is needed.

For the past 5-6 years the Southeast Fisheries Center, National Marine Fisheries Service has been establishing a data bank on the available chemical and nutritional data on fish and fishery products. To date, the bank contains data from approximately 1600 references. In general, on a particular aquatic animal, the data are quite fragmentary, especially for the vitamin, mineral and lipid components. To make this nutritional information more meaningful and useful, it is necessary to augment it with the following scientific information.

Proximate Composition

If you critically examine the proximate composition data in the commonly used U.S.D.A. Handbook #8, you will learn that the values are oftentimes based on the results of 1 or 2 analyses. The size of the fish, physiological status and seasonal variations are not considered in the values. For each commonly marketed seafood there should be a mean, range and/or standard deviation of observations used to calculate the above statistics. I understand that more data will be considered in the new edition of Handbook #8 now under development. (It will be available in another year).

More often than not, the data reported in the literature is on raw seafood. How often is raw fish eaten? Have you considered this? There are at least 40 different chemicals in seafood muscle. In the presence of heat and moisture, some chemical reactions or changes must take place. Consequently the flesh we eat can be different in composition and nutritive value than the original raw flesh. There's a scarcity of these data. Some effort should be made to identify the changes that occur when seafood is boiled, baked, panfried, and deep-fat fried. So often, the nutritive value of prepared, ready-to-eat seafood is assumed to be the total of the raw products in the recipe or formulation. That's not true, in fact, not even scientific.



### Lipoid Components

As we all know, the oils in seafood differ from vegetable oil, in that, they contain fatty acids with more than 18 carbons and up to five or six double bands. It has been assumed that marine oils function somewhat like the unsaturated vegetable oils, overlooking the longchained polyunsaturated fatty acids. It is recognized that each fatty acid has a characteristic metabolic pattern. Therefore, a question arises as to how animals, including man, metabolize the C20, C22 and C24 fatty acids. In a limited study, Borgman was able to show the differences in the function of marine and vegetables fats. He noted that he could dissolve the gallstones in the experimental animal, as well as prevent their formulation when the diet included cod liver oil, the vegetable oils only prevented the formation.

For the commonly marketed seafoods, we need to know more about the variation in composition of fish oils within species and between species. Changes that occur to the oils in the flesh when it is processed, such as canned, dried, frozen etc., also, what happens to the lipoid fraction when it is prepared for a meal? These data would make a significant contribution to the composition tables.

Nutritionally we should also know how these polyunsaturated fatty acids found in fish oils are metabolized under normal conditions. In addition, what metabolic changes occur in the presence of antioxidants like Vitamin E, selenium, etc.? Do these long-chain, unsaturated fatty acids have any effect in pathophysiology of the heart and any other organ in the presence or absence of such factors as hypoxia, stress, alcohol, etc.? What is the biochemical and physiological mechanism involved in the effects of dietary fats - especially the long-chained highly unsaturated fatty acids - on the ratio of the high and low lipoproteins? This type of information would be especially important to the medical community.

There is a great need for reliable data in the amounts of the different steroids in shellfish in the composition tables as well. In the literature the values for cholesterol in oysters varied from 400 mgs to 40 mgs. Presently, the National Heart and Lung Institute accepts the latter. In a recent small survey, I learned that at a certain time of the year oysters are low in cholesterol and none of the samples were as high as 400 mgs. The values ranged from 39 to 218 mgs, with an average of 110 mgs. We need to further the cholesterol content of raw and cooked oysters as well as other fish and shellfish. There are indications that cholesterol will leach out during cooking.

### Vitamins

In the December, 1978 issue of the Marine Fisheries Review, there is a resume of the vitamins content of the edible protein of seafoods obtained from literature. Additional data, however are needed to fill the empty

spaces. Since many of the water soluble vitamins are destroyed during processing it is also necessary to accumulate data on cooked and/or processed seafoods. Since the vitamin content of fishery products is quite scanty, there is an urgency to obtain this type of data for composition tables.

### Minerals

There presently is great interest in the bioavailability of the various nutrients especially after the raw food has been processed or mixed with other food constituents to prepare a dish, in other words, their availability as the "food is eaten". This is a fertile research area and needs to be more fully investigated. For example, years under rather crude techniques, fish were analyzed for iron. The resulting data indicate that fish is high in iron. Very little research has been done in this area since then. The question arises--is the iron found in seafood flesh available? We really don't know. These investigations should not be limited to raw flesh only, but also to processed fish. The interrelationships between various elements and the effect of the minerals in the oxidation process of fatty acids is another area that needs close examinations.

A further important area of investigation is to find out how a catch should be handled on ship, on shore, in the plant, on the store shelf and in the home in order to maintain high quality and good nutritive value.

By this time, the question on everyone's mind is how can all these studies be monetarily supported? I fully realize there is only so much money presently or potentially available. I recommend you consider the following options: (1) can an ongoing technology program be extended to include the development of needed composition data; (2) as ongoing research is phased out, can it be replaced with a nutritional research and; (3) can funds be reallocated to include nutritional and chemical studies.

In my opinion all these options can be considered seriously in planning research programs that are concerned with foods, in general, and/or fishery products.

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## FISH FLAKES OFFER EXPANDED FINFISH PROCESSING AND IMPROVED SEAFOOD USES

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### INTRODUCTION

This conceptually oriented project is designed for rapid transfer of observations and ideas to potential users. Its planning is based on equal time between laboratory and plant trials. Processor members of the Association have exerted input as it concerns developing multipurpose plants to eliminate seasonal operations and to diversify products being produced. In North Carolina this approach is especially applicable to crab picking, and scallop shucking operations.

The aim is to use varied sizes and kinds of finfish to produce appetizing meat flakes, free of skin and bone, and functionally effective for further processing, or for recipes prepared at home. Suitability for commercial or home use depends upon using fresh or frozen raw materials of the best quality, and employing processing conditions which assure firm texture, and attractive flavor and odor. Bland flavor and odor notes achieved by substantially removing body oils and avoiding oxidation has received some emphasis in recent months.

Most important in defining objectives is the effort to produce fish flakes with as much gustatory acceptance as has been achieved by various forms of canned tuna. In addition to this desire to produce something which can have wide acceptance as the chief ingredient it is also known that these products can perform functionally in improving the meats of crab, shrimp and clams in various commercially formulated products.

As part of this effort to retain the naturally pleasant properties of fish meats it seems likely that aqueous extracts, sometimes lost in processing, should in all or part be returned to the meats. A practical minded man named Denys Papin recommended this in the year 1681, having followed closely the work of the famous Robert Boyle who wrote only in Latin. Papin (11) described his pressure cooker experiments on animal tissues to the Royal Society, stating "that it would not be improper now to make upon the Subject a separate Treatise in the vulgar Tongue for the use of Housekeepers and Tradesmen as may have occasion for it!"

Another old approach employed in this project can be attributed to Scandinavian lumbermen who are believed to have brought the Wisconsin Fish Boil method to the shores of Lake Michigan 100 years ago (9). This

method involves dropping dressed fish in rapidly boiling strong saline solution which through elevated boiling point keeps fish intact without imparting salty flavor.

The first commercial pack of canned tuna was produced in 1907 by A. P. Halfhill of San Pedro, California in an effort to find a canned fish product to replace sardines. Although there have been many processing improvements as described by Gillies (6), the basic process still consists of precooking eviscerated tuna in direct steam at temperatures only a few degrees above 212°F (100°C) to accomplish the loosening of skin and reduced adherence of meat to bones, followed by cooling and hand separation of the light meats for canning.

Butchering methods have been directed at transverse and longitudinal cuts which facilitate removal of light meat portions and may indeed assist in the reduction of body oils. Fat contents in the meats of various tuna species are at times relatively high, but the meats after precooking are rather low as indicated by Adams (1) values for fats in tuna packed in water, and the Geiger (5) data shown in Table 1.

<u>Species</u>	<u>Moisture</u> (%)	<u>Protein</u> (%)	<u>Fat</u> (%)
Yellowfin (Large)			
Light Meat	66.6	32.2	0.6
Dark Meat	66.7	31.0	1.3
Yellowfin (Small)			
Light Meat	67.1	31.0	0.7
Dark Meat	66.7	27.6	2.6
Skipjack			
Light Meat	67.5	30.0	0.3
Dark Meat	66.4	28.7	2.2
Albacore			
Light Meat	68.1	30.0	1.2
Dark Meat	67.4	28.9	2.5

TABLE 1. Composition of Precooked Tuna Meats

The light meat of mullet becomes quite bland if subjected to steps which appreciably reduce fat levels. Waters (3) described a canned mullet pack prepared by NMFS which involved brining the dressed fish, subjecting it to live steam, separating the light meat and canning in brine solution. The staff preferred it to canned tuna in appearance, flavor and juiciness, even after 6 months storage.

The economics of using only prime parts of fish for a consumer product depends upon effective marketing and ability to justify a relatively high price. In this regard it is important to note that in many of the further processing operations the end products may benefit by use of both light and dark meats.

The tuna industry effectively uses some of the dark meats and various waste materials in pet foods or fish meal manufacture. Earlier it was mentioned that the aqueous extracts of fish would improve the meats. The economic advantages of almost complete use of a fish are well known to the menhaden industry which converts the raw material into fish meal, condensed fish

solubles and fish oil. One hundred tons of menhaden supplies about 12.6 tons of protein to the fish meal and about 4.0 tons of protein to the condensed fish solubles, the latter product derived from aqueous extracts of the fish (4).

In much the same way, when gilled and gutted bluefish, croakers, mullet, or grey trout are subjected to boiling water extraction followed by separation of the meat flakes, it is found that the ratio of protein in the flakes as compared to the level in the aqueous extract is about 4 to 1.

## MATERIALS AND METHODS

### Butchering techniques

X-ray photographs of several species helped pinpoint problem areas and can be usefully applied for training plant personnel. Angel et al (2) described removal of skin from frozen fish by immersion in boiling water. Ammermon, U. S. Patent 3,706,333, describes skinning of catfish, preferably headed, eviscerated and frozen, then placed in hot caustic bath for 1-3 minutes followed by rinsing and neutralizing in acid bath, eg. acetic or citric acid. Lindbladh, British Patent 1,463,262, is based on contacting the fish with acetic acid followed by water rinse to remove skin.

Lapeyre, Canadian Patent 956,066, suggests butchering frozen whole fish including its subdivision into a plurality of cross sections followed by subdividing the cross sections into frozen segments, the junctions between which are along lines generally parallel to the longitudinal axis of the fish and of its skeletal structure.

Trials have involved a variety of ways of cutting mullet, trout, croakers and bluefish to determine the effect of precutting and orientation of body parts upon quality and composition of fish flakes. Large, 9 to 12 pound, bluefish have been handled under pilot plant conditions to arrive at estimates of how the options bear upon labor costs and other factors.

The effect that skinning and selective cutting can have upon fat content and shelf life of grey trout is emphasized by Miller et al (10) based on analytical data determined by Gates. Fat contents of components were: Headed and Gutted - 6.28%; Fillet, skinned - 4.01%; Skin and Underlying Fat in the fillet area - 11.54%; Abdominal Cavity - 12.84%. The belly flaps frequently contained more than twice as much fat as the meaty portion of the fillets.

### Fish Blocks

Imported fillets of large fish have frequently been flaked and used as ingredients in commercially produced stuffed crab, crab cakes and other precooked seafood products. In preparing to produce fish flakes the processor must weigh every raw material option, including the relative merits of using species which are close at hand vs those which are imported.

Another consideration is the possibility of preserving more of the larger domestically caught finfish in block form. Ryan (12) describes the

process for producing fish blocks, United States consumption in 1976 being about 400 million pounds with only 0.6% produced in this country.

Fish block imports in 1977 were 385 million pounds (15) with cod representing 53.2%, pollock - 21.5%, haddock 8.0%, whiting - 5.8%, and Greenland turbot - 1.2%. Blocks from minced fish were 4.8% of total imports.

Fillet blocks of cod, pollock and haddock, each measuring 19" x 10" and about  $2\frac{1}{2}$ " thick were obtained from Iceland. Literature concerning these blocks indicated the protein and fat contents to be, respectively: 18.5% & 0.3% for cod; 20.1% & 0.7% for pollock; and 18.9% and 0.2% for haddock.

### Processing alternatives

A method readily adaptable to crab plants involved placing dressed fish on trays and exposing the materials to steam at 15 p. s. i. for the time required for internal temperatures to reach at least 160°F (71°C). Obviously the exact cooking condition selected depends upon subsequent handling and what is required for absolute safety. Excessive cooking reduces moisture contents to lower than desired levels, tends to increase transfer of protein to the liquid (condensate) phase while the level of retained fat remains about the same.

Lassen (8) investigated the effect of varying exposure of tuna muscle to steam. He reported that the moisture dropped from 71% in the raw meat to 66% when cooking was excessive.

Two methods of extracting gelatin and other substances from finfish heads and frames were conducted in the laboratory in order to produce material for blending with fish flakes. Both involved boiling the raw materials in a limited amount of water, followed by (a) separating the liquid by means of a Carver press, then separating the oil, aqueous phase and suspended solids by centrifuging, or (b) draining the liquid away from the cooked raw materials by gravity and allowing it to cool slowly to 40°F (4.4°C) in a cylindrical container. After a few hours the semi-solid fat was readily removed from the solidified aqueous material which can be used, with the exception of the sediment portion at the bottom. Other approaches being considered are based on gelatin technology involving the bones of land animals (13).

Cooking one pound fish such as dressed mullet, grey trout and bluefish by direct exposure to boiling 8% salt (NaCl) solution indicates that about 15 minutes is required. The fish retain a firm structure while being rinsed in fresh water, and the separations are readily accomplished.

The fish blocks were sampled by cutting (while still frozen) transversely into strips measuring  $2\frac{1}{2}$ " x 10" x  $\frac{1}{2}$ ". These were immediately placed in the boiling brine solution, the cooking requiring 5 minutes. After rinsing in fresh water the meats were mildly salty.

Another option for direct boiling of dressed fish, or fish block strips may involve the use of the aqueous extracts of the heads and frames.

Small scale trials have indicated such advantages as effective heat transfer to the raw meats, the recovery and recycling of solids which would otherwise be lost to the meats, improved flavor, and the possibility that the gelatin matrix will improve shelf life in frozen storage.

Shown in Figure 1 is an arrangement which might be used with either saline solution or aqueous extract as a heat transfer medium. If operated with salt solution there would have to be controlled input of concentrated brine to make up for the diluting effect of condensate. With either liquid there would have to be constant removal of fat and probably a method of evaporation for control of solids at the correct level. Obviously, in using aqueous extract the use of fresh water sprays following cooking would have to be eliminated.

#### Lipid determinations

Recent work involved use of a simplified version of the Bligh and Dyer Method AOAC 18.043 through 18.045 (14) and the the method of Ke et al (7) for determining free fatty acid. Determination of iodine value by the Hanus Method was included as part of the protocol. These rapid methods (16) will assist in monitoring what is accomplished by way of reducing lipid content through various processing methods, and some of the side effects of the cooking, cooling and handling techniques.

#### RESULTS

The experimental work demonstrated the importance of selecting butchering options which consider the shape, size and skeletal structure. The angle and direction of cutting finfish, and fish blocks, may greatly influence the characteristics of fish flakes, including the final lipid content.

The importance of correctly freezing raw materials intended for fish flake manufacture cannot be overemphasized. The method of storing the smaller sizes of finfish in the round, as described by Miller et al (10), is probably satisfactory, but ice glazing of large fish without suitable protection from mechanical damage is questionable. The conversion of such fish into block form is an obvious possibility.

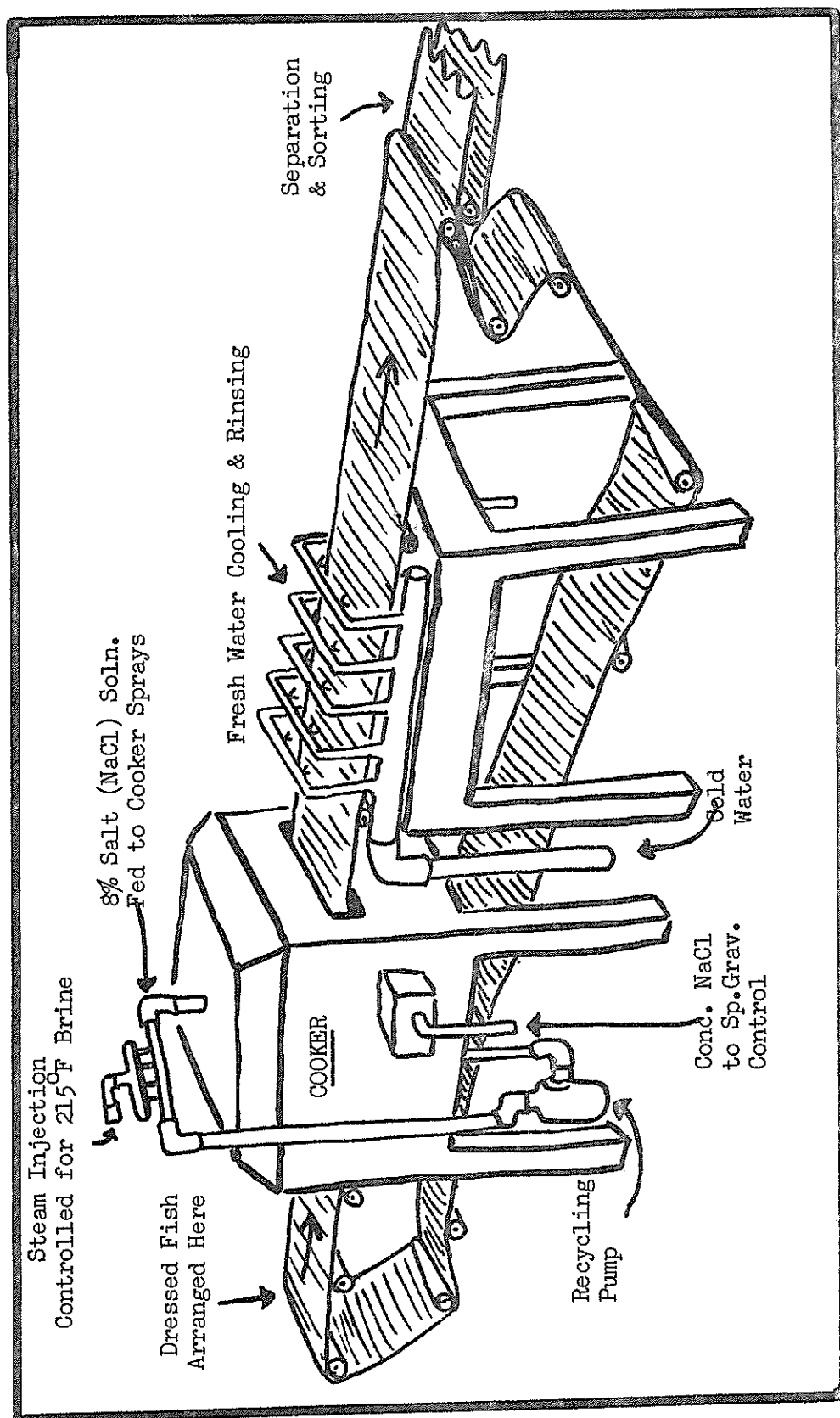
Cooking local species of finfish, or strips cut from fillet blocks in boiling brine produced flakes with promising characteristics. Cooking trials involving use of aqueous extract of heads and frames produced favorable results, especially from the standpoint of removing lipids from the meats.

Monitoring methods for measuring the effectiveness of the cooking method from the standpoint of lipid content, and other changes which may occur in subsequent handling were tried and considered **suitable** .

#### DISCUSSION AND CONCLUSIONS

Processor members of the Association continue to show strong interest in undertaking fish flake manufacture and the further processing into a diversified product line which will then become possible.





**FIGURE 1** PROTOTYPE COOKER FOR FISH FLAKE MANUFACTURE

An important part of the structuring of this project is its ability to initiate cooperative effort with processors at any time that the need is made evident. The first to make use of this opportunity was Mr. Tom Caroon of Oriental, North Carolina who worked with the investigators, made useful suggestions, and at the same time developed methods for his individual use. His two plants (Riverview Crab Co. and Tom Thumb, Inc.) are now producing fish flakes and an excellent stuffed clam product, containing fish flakes, on a commercial basis.

Fish flakes having good texture and ability to contribute bland, pleasant fish flavors and aromas can be applied in hundreds of recipes, as has been done with tuna. There is no doubt, based on numerous observations, that finfish meats can be mixed with shrimp, clams and crab meat in numerous formulations to make these seafoods taste better. The use of aqueous extracts of the heads and bones of finfish, introduced as part of the fish flakes, or as a separate ingredient, can also serve to improve flavor.

#### ACKNOWLEDGEMENTS

This project is supported by Gulf and South Atlantic Fisheries Development Foundation, Inc., Tampa, Florida in cooperation with several members of The North Carolina Fisheries Association, Inc.

Marine Chemurgics provided the research support and pilot plant work described herein.

Mr. Jerry Kiser, Kiser Brokerage Company, Charlotte, N. C. arranged to have the fish blocks supplied and delivered for experimental work. The blocks were furnished by Iceland Products, Inc., produced under the brand name of "SAMBAND OF ICELAND". The composition data on cod, pollock and haddock is from the same source.

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THE DETERMINATION OF OPTIMAL COOKING TIME  
AND TEMPERATURE OF SHRIMP IN MICROWAVE COOKERY  
AND CONSUMER ORIENTATION ON THE SUBJECT

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This project was concerned with 3 objectives:

- 1) to determine the effects of microwave cooking on the quality characteristics of shrimp
- 2) to develop the technology and formulation necessary for the commercial production of microwavable shrimp dishes and
- 3) to develop, test and compile recipes into a consumer publication.

Four counts of shrimp, 43/50's, 31/35's, 21/25's and 16/20's were used in determining cooking curves in order that optimum time and temperature settings be reached. Each shrimp size was randomly selected and placed in a single layer in a petri dish. Two trials were run for each count: one consisting of 5 shrimp per petri dish and the other trial had 8 shrimp per petri dish. The petri dish was placed in the microwave oven for a given time and then immediately removed. The internal temperature of each shrimp was taken with a thermocouple unit in the thickest part of the shrimp tail for a temperature recording, removed and placed directly into another shrimp until all were recorded. (The various temperatures were recorded on the Leeds & Northrup Speedomax Recorder). Procedures were repeated at specific time intervals until the temperature leveled and a cooking curve was established. From the graphs, a breaking point for each curve was reached. The breaking point is that area at which leveling occurred and will be a critical value for use in further analysis. From this, we arrived at optimum time and temperature settings. However, the points on each side of the breaking point may eventually prove to be the optimal time needed.

Size 43/50

An 8.4 cm petri dish was used in the microwave cooking of 43/50 count shrimp. As was expected, these small shrimp had a short cooking time and reached high temperatures quickly. Beginning with zero seconds for room temperature and proceeding to 60 seconds at 5 second intervals a cooking curve was established. The breaking point was 30 seconds at 94°C for 5 shrimp per petri dish, while the breaking point for 8 shrimp per petri dish was again 30 seconds but at 84°C.

### Size 31/35

A 13.97 cm petri dish was used for the 31/35 count shrimp. Letting zero seconds be room temperature and proceeding to 90 seconds at 10 second intervals, a cooking curve was formed. The breaking point for 5 shrimp per dish was 60 seconds at 99°C, while the breaking point for 8 shrimp per dish was 70 seconds at 91°C.

### Size 21/25

A 13.97 cm petri dish was used for the 21/25 count shrimp. Letting zero be room temperature and proceeding to 96 seconds at 10 second intervals, a cooking curve was formed. The breaking point for 5 shrimp per dish was 50 seconds at 86°C, while the breaking point for 8 shrimp per dish was 80 seconds at 90°C.

### Size 16/20

A 13.97 cm petri dish was used for the 16/20 count shrimp. Zero was again room temperature. Beginning with 30 seconds and proceeding to 100 seconds at 10 second intervals, a cooking curve was formed. The breaking point for 5 shrimp per dish was 40 seconds at 80°C and the breaking point for 8 shrimp per dish was 80 seconds at 84°C.

TABLE OF RESULTS

<u>Count</u>	<u>Shrimp per dish</u>	<u>Time (seconds)</u>	<u>Temperature (C°)</u>
43/50	5	30	94
21/25	5	50	86
31/35	5	60	99
16/20	5	90	95
43/50	8	30	84
21/25	8	80	90
31/35	8	70	91
16/20	8	80	84

## CONCLUSION

An important factor in microwave cooking is the product arrangement in the dish and the dish location in the microwave oven. Most electromagnetic waves pass through the center of the oven with few waves penetrating the four corners of the oven. It was found that as the quantity of shrimp per dish increased, the cooking times must increase. Also, if the dish is overloaded, or the shrimp conceal each other, the two curves for the same shrimp size steadily rose and gradually reached a peak. The 8 shrimp per dish cooking curve remained below the 5 shrimp per dish curve. This illustrates well that the more shrimp per dish, the lower will be the peak at a given time setting.

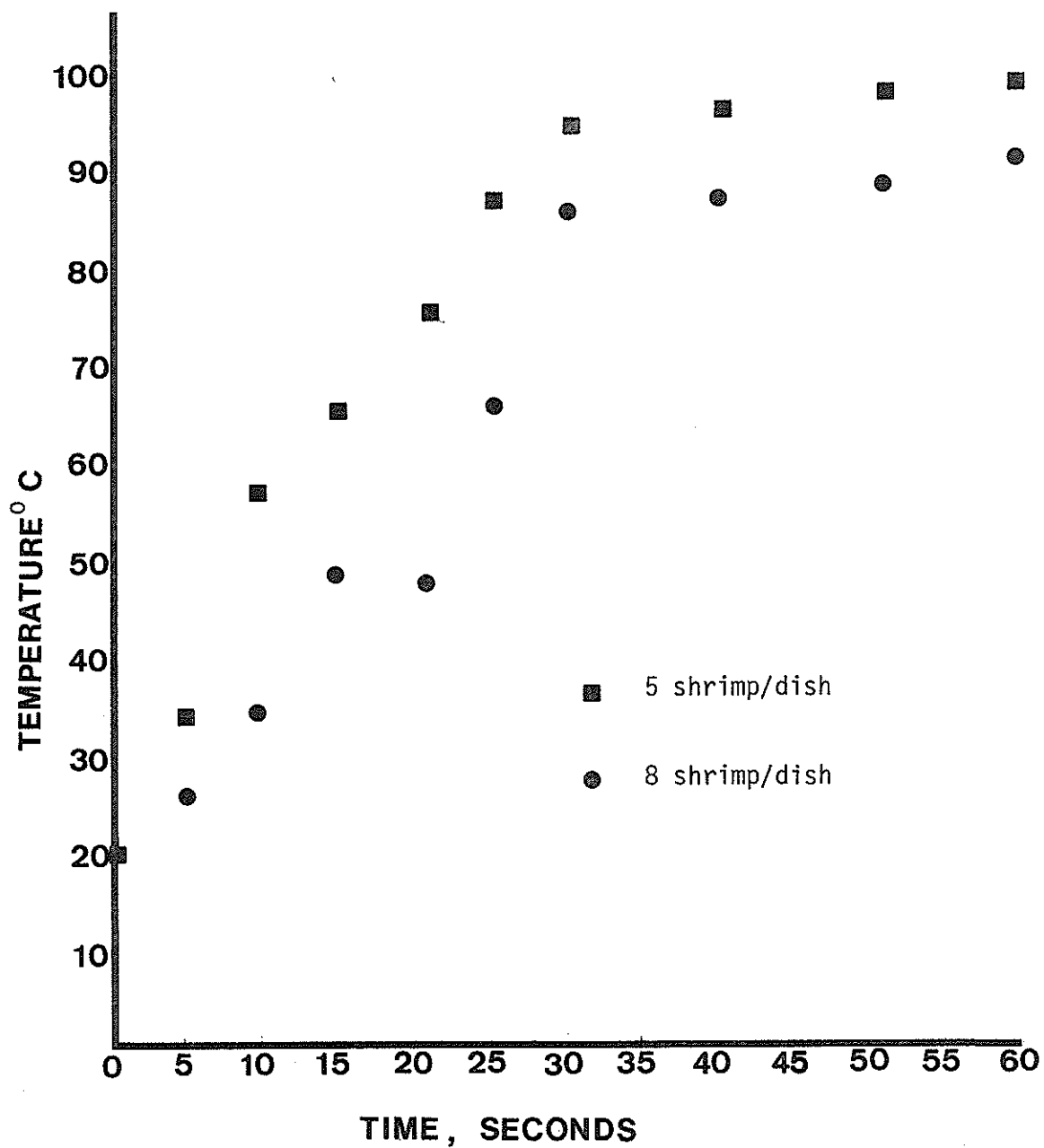
We will be able to use this information to make recommendations to commercial fast food chains who have indicated interest in selling microwavable shrimp dishes in their operations. With this in mind, seafood technology students developed formulations for microwave compatible dishes for commercial production. It was determined which recipes responded favorably to conventional precooking, freezing then microwaving. Samples of shrimp gumbo have been provided to a state food processor for evaluation of cost, formulation and suitability for marketing.

It is a well known fact that shrimp cooks quickly by conventional methods, but now via microwave cookery, shrimp can be even more of a convenient food item for 3 million microwave oven owners.

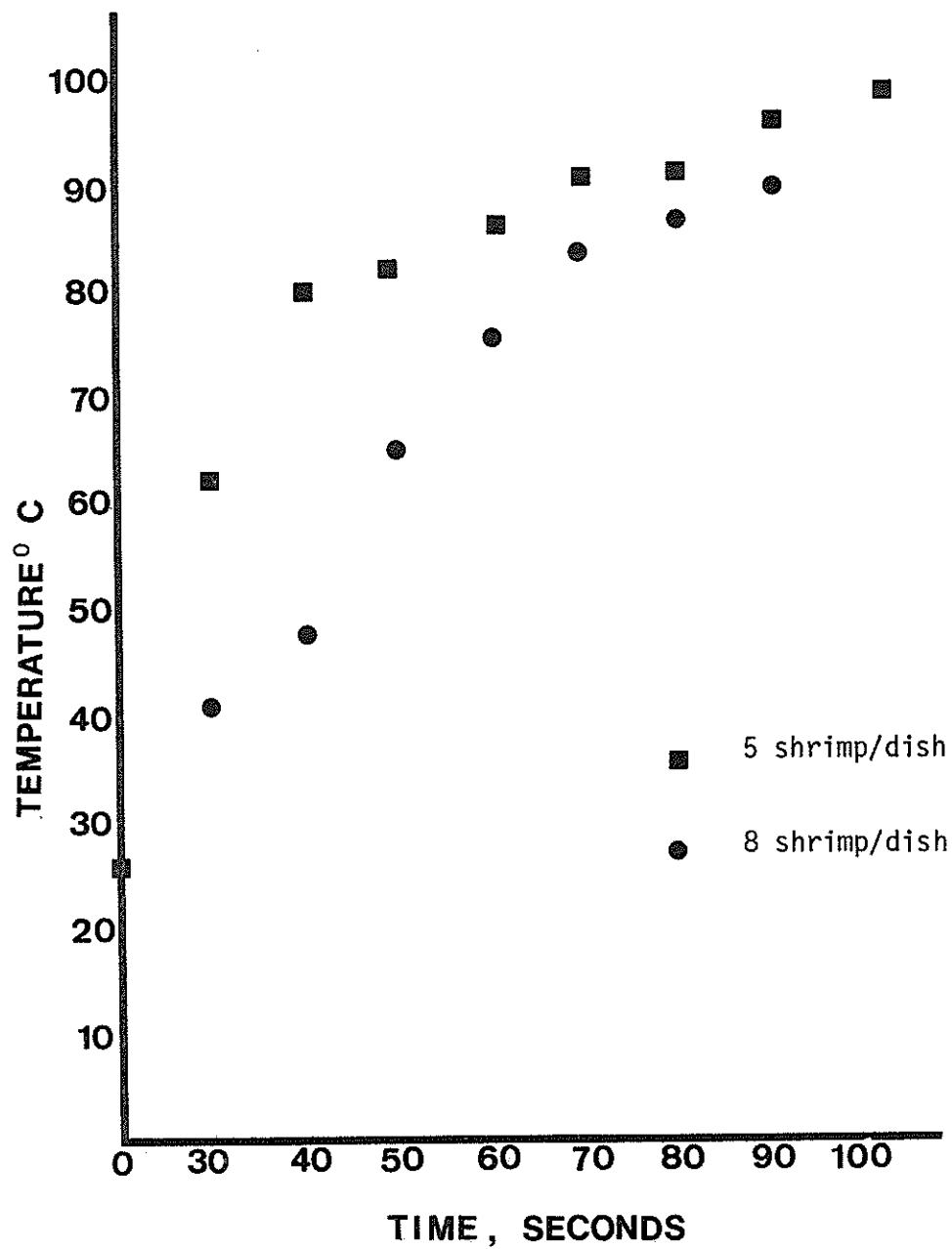
At the time this project began, with industry support, there was an excess of supply in inventories and imports. Inventories carrying over from the 1977 season plus imports placed supplies at about twice the demand. These conditions plus low catch rates were forcing many shrimp fishermen to cease operations. To encourage increased home preparation of shrimp, a consumer publication featuring shrimp cookery with microwave is being developed. The procedure involves developing, testing and evaluating recipes through an experienced test panel to determine acceptability of appearance, taste, aroma and overall satisfaction. Categories of the 60 recipe cookbook include hors d'oeuvres, salads, side dishes and entrees.

Distribution will be statewide to consumers through various means and available through seafood retail outlets at the point-of-purchase and possibly through microwave distributors.

It seemed that a logical approach to stabilizing shrimp marketing through the development of convenience dishes was through the utilization of microwave cooking. With increasing interest in nutrition, thus great seafood consumption coupled with the popularity of the microwave oven, interest in time and energy savings - it is believed that shrimp will obtain a stronger position in the consumer market. This task will be accomplished by making shrimp a more convenient food item to the consumer through industry cooperation and consumer education.

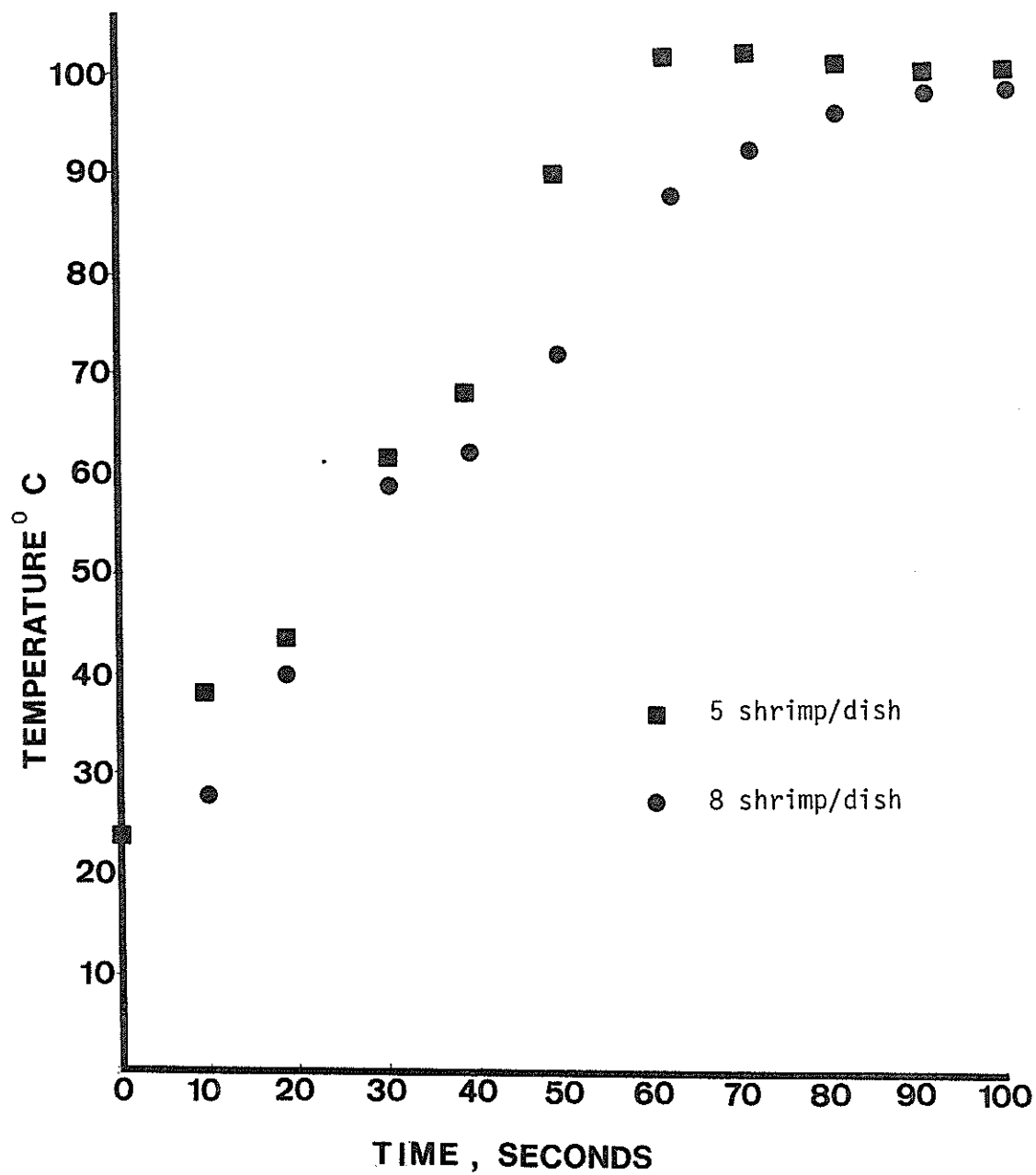


Microwave Cooking Curve For 5 & 8 43/50 Shrimp in 5 1/2" Petri Dish

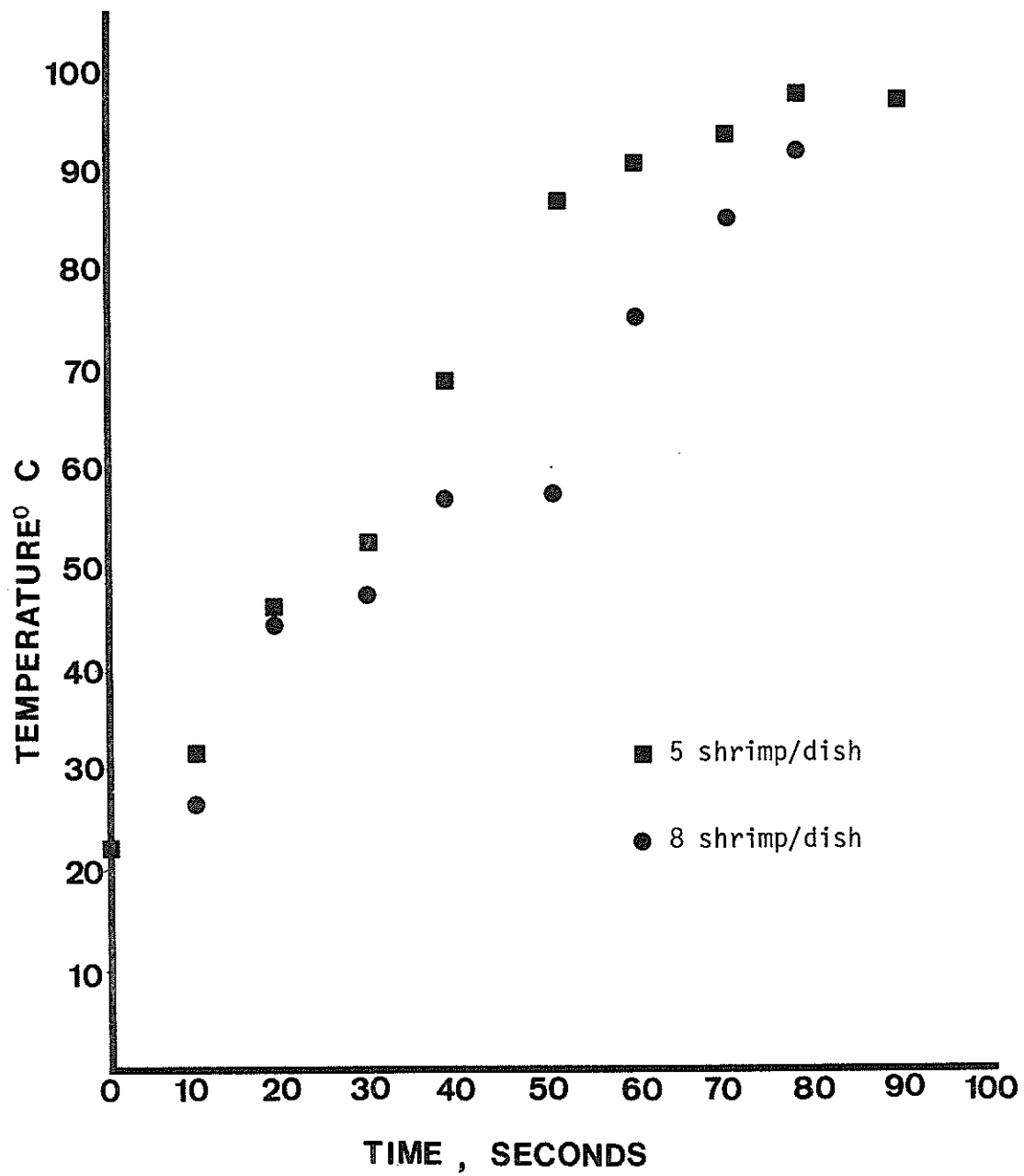


Microwave Cooking Curve For 5 & 8 16/20 Shrimp in 5 1/2" Petri Dish





Microwave Cooking Curves For 5 & 8 31/35 Shrimp in 5 1/2" Petri Dish



Microwave Cooking Curve For 5 & 8 21/25 Shrimp in 5 1/2" Petri Dish

THE BIOLOGY AND FISHERY OF THE QUEEN CONCH  
(STROMBUS GIGAS): A REVIEW

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The queen conch (Strombus gigas) is an important molluscan fishery resource throughout the Caribbean. Abundance, ease of collection, and value as a protein source (15) has insured its significance since man first settled in the Caribbean. Archaeological evidence shows that the queen conch was an important resource for Caribbean Indians before Columbus' discovery of the New World. For many years, dried and live conch were shipped aboard sailing schooners throughout the Caribbean, providing a valuable dietary staple. Although queen conch has long been an important resource, it has become even more significant in the last decade due to: (i) population growth, (ii) increased tourism, and (iii) development of export markets for frozen conch meat. This report presents a summary of what is known about queen conch biology and fisheries.

METHODS AND MATERIALS

The information presented here was compiled under the auspices of the Monroe County (Florida) Marine Resource Inventory Program. Sources of information included: (i) scientific literature, (ii) fishery statistics, and (iii) personal communications with queen conch biologists and fishery experts. The author gratefully acknowledges Mr. Willard Brownell, University of New Hampshire Marine Advisory Program, and Dr. Kenneth Haines, University of Texas, for their assistance.

BIOLOGY OF QUEEN CONCH

The term "conch" (pronounced "konk") is commonly used to refer to a diverse collection of marine gastropods, or snail-like animals. However, the tropically occurring queen conch is easily recognizable by the large, flaring lip and rich shades of pink,

yellow and orange in the aperture. Abbott (1) described its range as southeast Florida and the entire Caribbean area south to Brazil. It is often abundant in shallow areas where attached algae and seagrasses, the food of queen conch, occur.

Little (17) described the anatomy of the queen conch and also examined ionic regulation (18). There have been several studies of strombid conch behavior (4,5,21,22). Randall (24) presented the most thorough compilation of data on its biology to date.

Spawning activity occurs in the warmer portion of the year (9,12,24) although egg masses have been observed in many parts of the Caribbean throughout most of the year (Willard Bronnell, personal communication). The eggs are fertilized internally by males (6,25), and the number per mass has been estimated at between 313,000 and 485,000 (12,24,25). Females apparently produce more than one egg mass during a spawning season (24,25).

The larvae emerge from the egg case after five days (9,12). Although larvae continue to feed on phytoplankton, they assume a primarily benthic behavior at 18-21 days, with transformation to the adult form at 28-33 days (9).

Several investigators have examined queen conch growth rates (Table 1). At an estimated age of 2.5 years, approximately 17.5 cm to 20.5 cm in length, the conch starts formation of the broad flaring lip characteristic of the adult form (6,15). In terms of meat production, this is the optimum size to harvest (15). Sexual maturity is attained after the flaring lip is well developed (6,7,15).

Table 1. Size of queen conch at one, two, and three years of age.

Age (years) and Length (cm)			Method of Calculation and Geographic Location	Reference
1	2	3		
8.8	12.6	18.0	Size frequency distribution of natural population (Puerto Rico)	6
7.6	12.8	18.0	Size frequency distribution of natural population (Venezuela)	9
10.8	17.0	20.5	Von Bertalaffy growth curve analysis of Randall's (24) tag/recapture data (St. John, U.S.V.I.)	6,9
No data	17.0	18.6	Von Bertalaffy growth curve analysis of nine populations using tag/recapture data (Cuba)	3 figure 4

Berg (6) estimated the average life span to be approximately 6 years. Randall (24) reported 22 species of fishes and invertebrates which consume queen conchs. However, once queen conchs develop the thicker, flaring lip shells they are rarely preyed upon (6).

## THE QUEEN CONCH FISHERY

### Harvesting and Processing

The importance of the queen conch has been well documented (8,14,19,20). Conchs constitute the second most important fishery in the Caribbean, after lobsters in the export industry and after finfish when it comes to local consumption (Willard Brownell, personal communication). Conchs are also taken for use as fish trap bait (11). The brightly colored shell is often sold for ornamental purposes and was once used in the manufacture of lime and porcelain (8,24). Occasionally, an irritating particle lodged between animal and shell results in production of a conch pearl. Although these pearls often have high market value, demand is limited since they fade with age.

Historically, sailing sloops 20-35 feet in length were used for harvesting. These sloops would sail to good fishing areas and several men would fish in small dinghies. The bottom was scanned through a glass bottom bucket until a conch was located. A long pole was then used to hook the conch and bring it to the surface. Fishing trips lasted 1-5 days, the fishing grounds not normally being more than one day sail away. Conchs were brought to port in the live well of the vessel, usually tied in bunches of about four to seven with palms strands through holes knocked in the lips of the shell. Conchs were also sun-dried or salt-dried for transportation.

Currently, small power boats have become popular although small sail boats are still used because of the added expense of an engine. Diving for conchs has become common. When a good area is located the fishermen uses diving mask and fins to retrieve conchs. By using either of these methods a fisherman can collect as many as 500 or 600 conchs per day (15,23).

The meat is removed by knocking a small elongate hole between the third and fourth whorls of the spire. A narrow, sharp blade is inserted in the hole, and the animal is cut free of its attachment to the central axis of the shell. A twisting motion can then be used to remove the animal. This is usually done by the fisherman before returning to shore. At the fish house the soft parts (eyes, viscera, etc.) are removed from the muscular foot. Often the tough dark skin is peeled away from the foot. The marketable meat yield is approximately one-half the weight of the animal after the shell has been removed (16). The waste may be sold as fish trap bait.

If the conchs are to be exported they are usually frozen. In the past, large quantities of conch were dried, before shipping. After beating conch meats until they were about one-half inch thick, they were hung in the hot sun for about three days. After such drying they would keep for about five or six weeks without risk of spoilage (23). However, due to the greater profit associated with export of frozen products to the U.S.A., export of dried conch is becoming increasingly uncommon, except in some inter-island trading (Willard Brownell, personal communication).

#### Current Status of the fishery

Conch meat has long been an important fishery resource in the Caribbean. Doran (13) documented export of dried conch meat from the Turks and Caicos beginning in the late 1800's. In 1943 alone, 3.9 million dried conch meats were shipped to Haiti. However, it has only been since the 1960's and early 1970's that queen conch has become a valuable fishery export product, primarily because of growth of an U.S. market for frozen conch meat (7,15,16). Growing population and increasing tourism have also been cited as resulting in growing demand (6).

In Belize, British Honduras, queen conch rose from 2.2% of the value of fishery export products in 1965 to 23.7% in 1976 (7). Pounds of meat exported rose from 50,000 kg in 1965 to a peak of 563,000 kg in 1972(7). Although export of dried conch from the Turks and Caicos declined during the 1960's to the point of being considered unimportant by the local government, introduction of frozen meat to the U.S.A. market in the early 1970's resulted in a dramatic increase of queen conch landings (16).

Unfortunately, there are indications that currently fished queen conch stocks are insufficient to meet today's demand. Although conch imports into the U.S.A. more than doubled from 212,400 kg in 1970 to 544,680 kg in 1975, in recent years imports have leveled off (Figure 2) in spite of rising prices. Blakesley (7) reported that the export price of conch meat in Belize rose from 0.27 Belize dollars per pound in 1965 to 1.50 Belize dollars per pound in 1976. In the Turks and Caicos, prices paid to fishermen rose from \$0.06 per conch in the 1960's (16) to a current (1978) price of \$0.18 per conch (Willard Brownell, personal communication).

Increased fishing pressure resulting from increased economic return in the fishery has resulted in many reports of stock depletion. In Belize, production has declined to 257,800 kg in 1977, less than half the production in 1972 (Figure 1). Also, fishermen have been complaining that only small conchs are available (7). Due to concern over stock depletion a closed season on conch from July 1 to September 30 has been imposed by the Belize Fisheries Department (Willard Brownell, personal communication). In Venezuela, concern over stock depletion resulted in restriction of fishing effort (10). In addition, there are reports of dwindling

Figure 1. Annual export of queen conch meat from Belize, British Honduras, 1965 - 1977 (7).

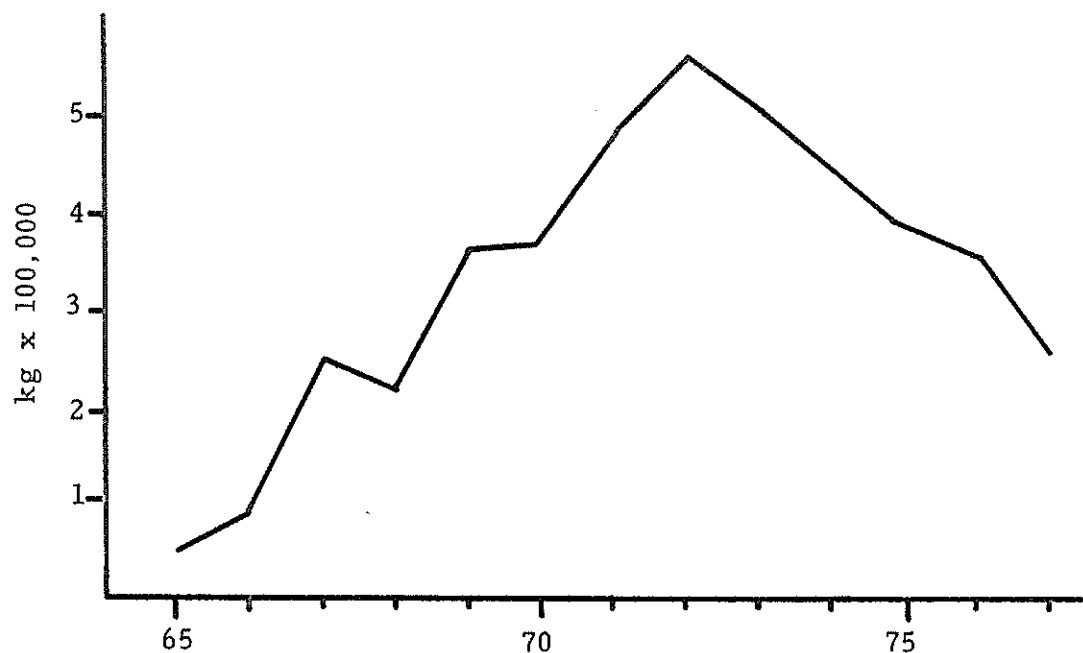
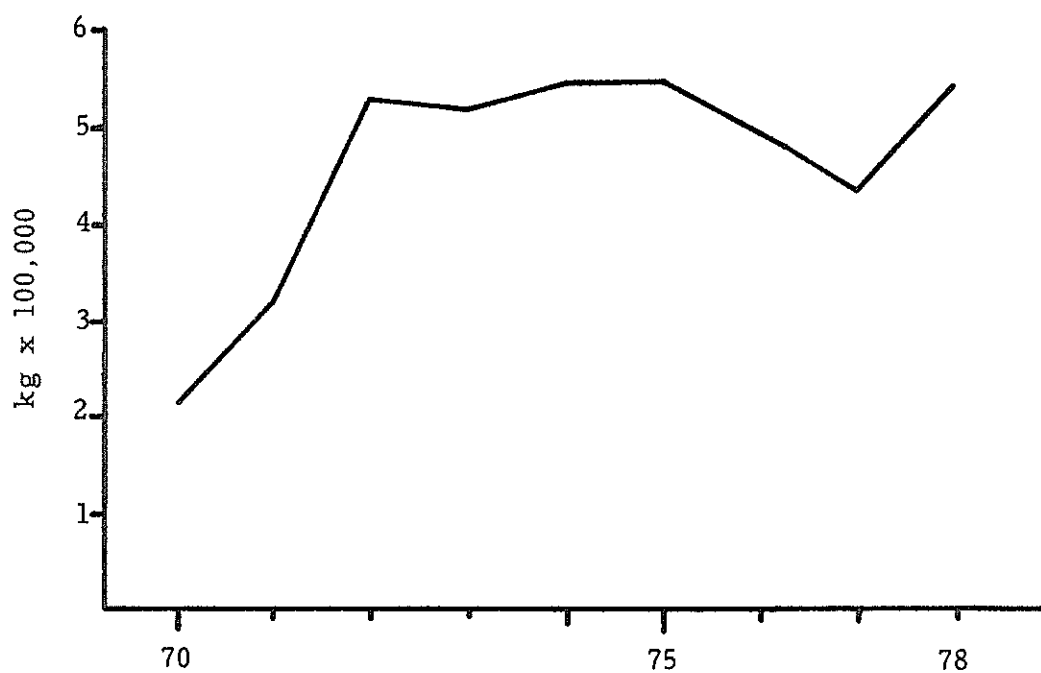


Figure 2. Kilograms of conch meat imported into the U.S.A. through Miami, 1970 - 1978 (Source: Market News Report, U.S. Dept. of Commerce, New Orleans, LA).



abundance in the Bahamas (8,11), the Grenadines and Puerto Rico (2), and the Turks and Caicos (16).

### CONCLUSIONS

Due to growing population and tourism, and the development of a lucrative market for frozen conch meat in the U.S.A., the queen conch has become an important source of revenue in addition to being an important food staple in the Caribbean. However, falling conch production throughout the region in spite of rising prices and increased fishing effort indicates that presently fished stocks are insufficient to meet demand. Overall production appears to be at a maximum, unless: (i) currently intensively fished stocks can be managed to stabilize falling production levels, (ii) new fishing areas can be located and utilized, (iii) conch mariculture and/or subsequent stocking prove to be useful for increasing production. Since conch is such an important staple food and is also bringing high prices on the export market, efforts must be made to save this fishery for the small-scale fishermen of the Caribbean.

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THE STABILITY OF ADENOSINE DEAMINASE AND ADENOSINE  
MONOPHOSPHATE (AMP) DEAMINASE DURING ICE STORAGE OF  
PINK AND BROWN SHRIMP

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Quality changes that occur in iced shrimp during storage are considered to be the result of combined action of tissue enzymes and microbial activity. The importance of ammonia formation during spoilage has been evident for a long time since many of the quality indices used for shrimp evaluation are based on ammonia production.

The importance of enzymatic ammonia production by shrimp tissue enzymes was shown by Yeh et. al. (1978). They found that the important enzymes were adenosine deaminase and AMP deaminase. The activity of these enzymes during initial post mortem storage amounted for more than half of total ammonia production.

The purpose of this study was to investigate the stability of AMP deaminase and adenosine deaminase in penaeid shrimp during ice storage. In addition to enzyme stability determination, the shrimp were also analyzed for total plate count (TPC) and total volatile nitrogen (TVN) and sensory evaluation.

#### MATERIALS AND METHODS

Pink and brown shrimp were obtained from fishing boats on the Texas coast. After deheading and washing the shrimp, they were stored in ice chests well mixed with ice. Samples were taken on a daily basis.

Protein was determined according to Kjeldahl nitrogen (AOAC, 1975). Total volatile nitrogen by the method of Cobb et. al. (1973). Ammonia was determined by the colorimetric method described by McCullough (1967). Total plate count estimated by the agar pour plate method (APHA, 1970).

Extracts for enzymes assay were prepared according to Lee (1957). Enzyme activity determined according to method of Kalckar (1947).

The shrimp were organoleptically evaluated by the boil in bag method. Shrimp were treated in closed plastic bags in order to retain volatiles released during heating. These samples were served in cooled bags and the taste panelist were asked to evaluate the shrimp for odor,

flavor, texture and desirability.

## RESULTS AND DISCUSSION

The stability of pink and brown adenosine deaminase is shown in Tables 1 and 2 respectively. Adenosine deaminase from pink shrimp was stable during the first week of ice storage, followed by a progressive decrease in activity. Brown shrimp adenosine deaminase exhibited the same phenomena as pink shrimp adenosine deaminase. The progressive decline in activity in both pink and brown shrimp adenosine deaminase was about 50% of the original activity after 9 days of storage.

AMP deaminase was found to be much more unstable than adenosine deaminase. Table 1 shows a 50% drop in activity after 4 days as compared with initial storage activity. This is in agreement with the findings of Dingle and Hines (1967), who also detected a similar decrease in crude cod muscle extracts. Flick and Lovell (1972) also found AMP deaminase to lose its activity in Gulf shrimp during ice storage. In this study, brown shrimp AMP deaminase showed an initial increase in activity, which was followed by a progressive decrease from the 4th day on.

This study confirms the presence of AMP deaminase in pink and brown shrimp; on the other hand, the enzyme was shown to be unstable and lost its activity during ice storage.

In addition to enzyme activity, the ice-stored shrimp were also evaluated for total volatile nitrogen, total plate count and organoleptically. Figure 1 and 2 shows enzyme stability, TVN, TPC and organoleptic evaluation on pink and brown shrimp respectively.

If spoilage as signified by the total plate counts can be divided into three phases (the lag phase, the log phase and the stationary phase), shrimp quality can be correlated to the following. During the lag phase, total plate counts range between  $10^2$ /g to  $10^4$ /g of shrimp; TVN values are below 20 mg N/100 g shrimp tissue. AMP and adenosine deaminases both are active but AMP deaminase loses about 50% of its initial value. Active enzymatic activity probably makes a significant contribution to ammonia production since total plate counts are relatively low. The organoleptic scores average 7.5, and the shrimp are of prime quality with regard to color, texture and juiciness. During the log phase, total plate counts are between  $10^4$ /g and  $10^7$ /g and TVN values begin to build up to 25 mg N/100 g shrimp tissue. AMP deaminase loses all activity while adenosine deaminase loses about 50% of its initial value. As a result of the increasing total plate counts and the loss of AMP deaminase activity, bacteria and adenosine deaminase are probably equal contributors to ammonia production. Organoleptic scores are between 5.0 to 6.0., and the shrimp are still of acceptable quality since there are no significant changes in color or flavor. Finally, during the stationary phase, total plate counts are in excess of  $10^7$ /g of shrimp and TVN values are more than 30 mg N/100 g shrimp. Adenosine deaminase is still active at a low constant activity of 0.10 to 0.05  $\mu$ mole/g/min. The shrimp become tasteless with the evolution of a pronounce ammonia smell. The high total plate counts and the loss of enzymatic activity indicate that bacteria are the major ammonia producers.

Table 1 - Stability of pink shrimp deaminases activity at 0-3°C,

Storage Time (Days)	Adenosine Deaminase*	AMP Deaminase**
2	100	100
3	100	75
6	95	32
9	35	5
11	32	0
14	30	0
17	19	0
20	21	0
21	13	0

Total activity is the number of micromoles of substrate deaminated per min. per gram of moist tissue at 25°C. Conditions for extraction and assay are given in the experimental section.

Results are given as percentage of the maximum velocity.

\* 12 mg/liter adenosine in 0.05 M phosphate buffer, pH 7.5.

\*\* 15 mg/liter AMP in 0.1 M sodium succinate buffer, pH 6.5.

Table 2 - Stability of brown shrimp deaminases activity at 0-3°C.

Storage Time (Days)	Adenosine Deaminase*	AMP Deaminase**
1	100	64
4	87	100
7	82	43
10	75	41
13	38	7
16	34	0
19	30	0
21	27	0

Total activity is the number of micromoles of substrate deaminated per min. per gram of moist tissue at 25°C. Conditions for assay and extraction are given in the experimental section.

Results are given as percentage of the maximum velocity.

\* 12 mg/liter adenosine in 0.05 M phosphate buffer, pH 7.5.

\*\* 15 mg/liter AMP in 0.1 M sodium succinate buffer, pH 6.5.

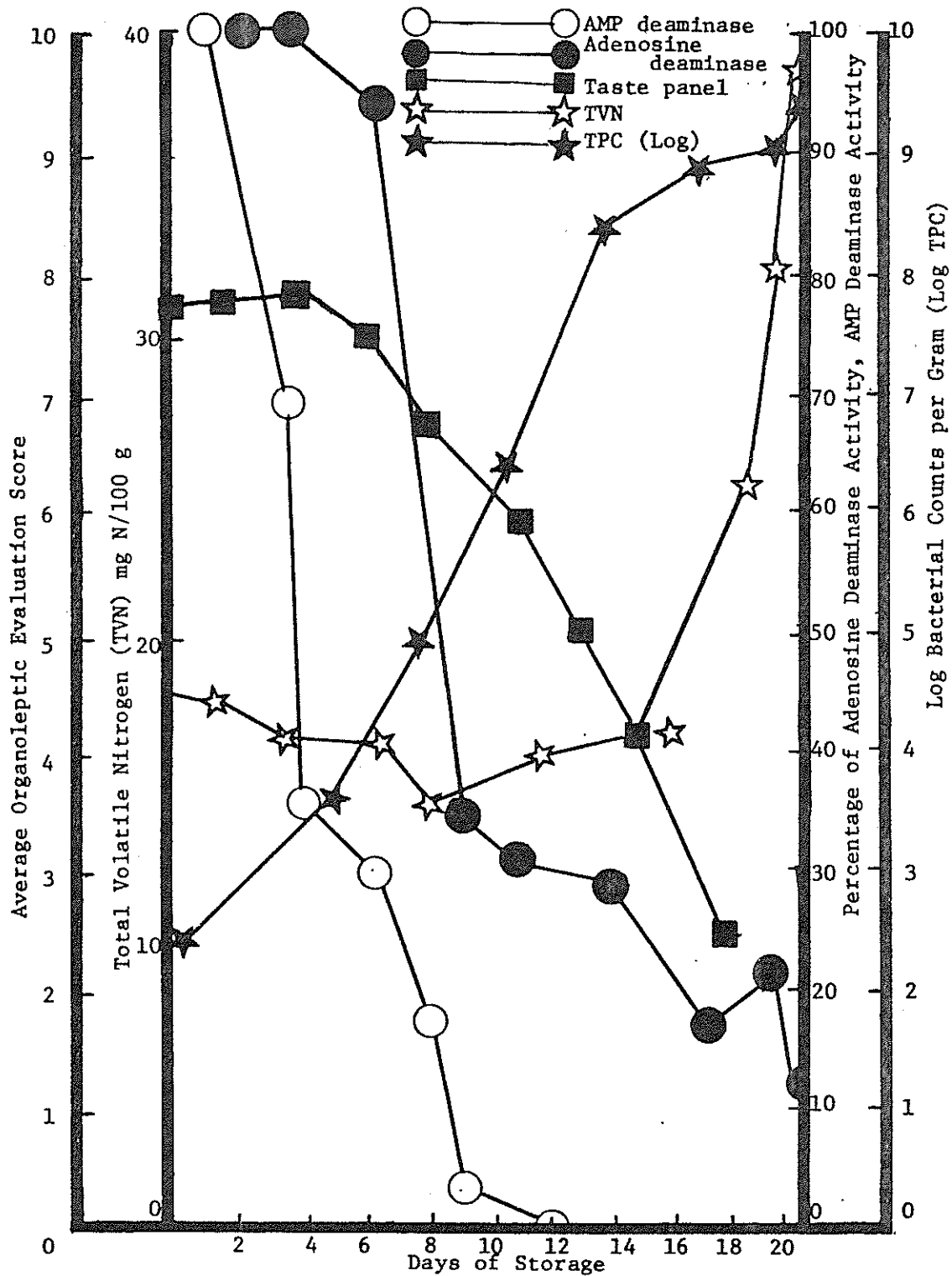


Fig. 1 - Effects of storage time at 3°C (38°F) on the adenosine and AMP deaminases activity, the agar plate counts, total volatile nitrogen (TVN) and the organoleptic evaluations carried out on a lot of pink shrimp stored in ice.

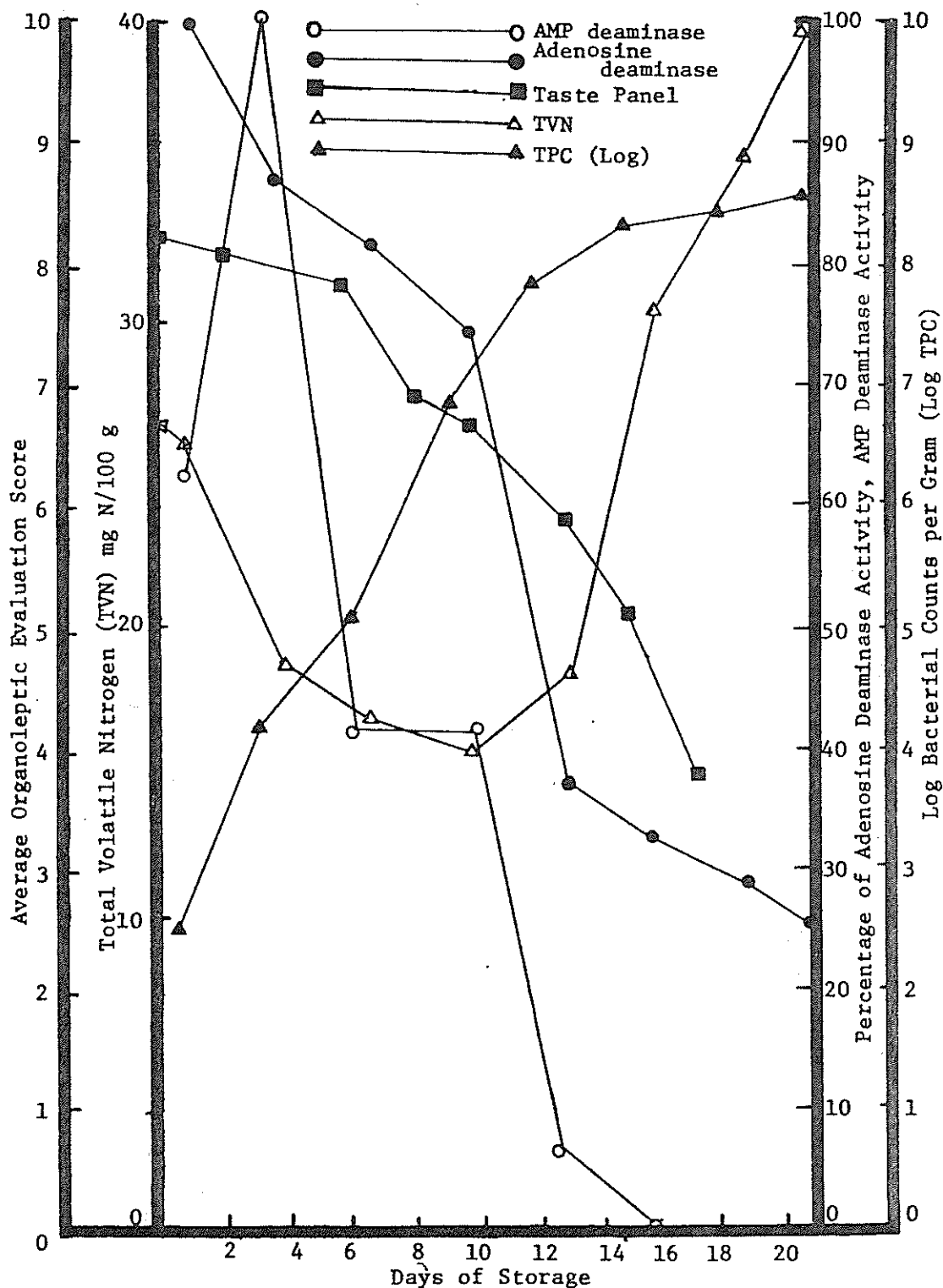


Fig. 2 - Effects of storage time at 3°C (38°F) on the adenosine and AMP deaminases activity, the agar plate counts, total volatile nitrogen (TVN) and organoleptic evaluations carried out on a lot of brown shrimp stored in ice.



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# THE EFFECT OF SALT, TRIPOLYPHOSPHATE AND SODIUM ALGINATE ON THE TEXTURE QUALITY OF FISH PATTIES

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As retail prices of conventional fish species have steadily increased all over the world fish has ceased to be an inexpensive item. As a consequence of demand exceeding supply fish processors will be faced with a tightening supply of conventional fish species. The new 200 mile fishing jurisdiction will probably increase the supply of non-conventional resources in the U.S., and an effort must be made to develop the technology necessary to put these new resources into the channels of commerce.

Recently, the underutilized scraps and frames of common fish and some of the non or partially utilized species (such as cod, sole, hake, and croaker) have been exploited to process minced fish products in the U.S., Japan, Denmark and other countries (7,3,5,6,8,11,12). Filleting finfish leaves a considerable quantity of edible flesh remaining on the frame. After filleting, the leftover flesh can be recovered by using a meat-bone separator to recover the minced fish flesh (4). The total yield of flesh removed with a deboning machine usually doubles or triples the yield of intact flesh removed by hand (5). Yield varies with the species of fish and the type of meat bone separator. Certain fish species which are too bony or too small to fillet efficiently can be fully utilized through the machine deboning process (4). The deboning machine permits the recovery of flesh from filleting wastes and has made possible the economical processing of previously unused species with low flesh yield (4,10). Fish which are underutilized due to their undesirable characteristics such as poor texture and flavor can be improved through chemical or physical treatment in the processing of the minced flesh (4,8,10).

There is wide variation in the quality of minced fish products. Various processing factors affecting quality include the deboning step, washing effect, chemical treatments (bleaching), the addition of salt, polyphosphate, alginate and/or spices, the cooking time and temperature storage conditions and fish species (1,4,7,10).

Fish and ham sausage have recently become popular in Japan. The sausage typically contains minced fish flesh with 10% pork fat, 10% starch, 2.5% salt, seasoning and preservatives (11). In the United States, Carver and King (1) developed fish frankfurters containing 76% fish plus added starch, oil and seasoning. Daley et al. (13) developed the "Sea Dog" which is a sausage-type product containing minced mullet, textured soy flour, tripolyphosphate and seasonings. Thus, minced fish represent a

potential source of inexpensive protein for human food if it can be incorporated into acceptable products.

In this study the black mullet species (Mugil cephalus) was used as the primary raw material for the development of a seafood patty. The purpose of this study was to develop a seafood patty from Florida under-utilized fish in combination with other ingredients, and to determine possible optimum combinations of the various major ingredients.

## EXPERIMENTAL

### Materials

Mullet (Mugil cephalus) were purchased fresh from Cedar Key, Florida, during February. Fish were transported on ice to Gainesville, Florida, where they were packaged in 5 kg bags and frozen at -30°F until used. Food grade sodium chloride (NaCl) was purchased from Morton Salt Company, Chicago, IL, food grade sodium tripolyphosphate (TPP) was obtained from the FMC Corp., Philadelphia, PA, sodium alginate (NaAlg.) was obtained from Kelco (Clark, NJ), calcium chloride (CaCl<sub>2</sub>) was purchased from Matheson, Coleman and Bell (Norwood, OH). Batter and breading mixes were purchased from North America Food Service, Chicago, IL.

### Minced flesh preparation

The fresh-frozen mullet were thawed at 35°F for 18-20 hours prior to use. They were scaled, cleaned, halved, packed on ice and stored in a 35°F refrigerator room overnight. On the following day the cleaned fish were passed through a deboner (Baader 694, Nordescher, Maschinenbau Lubeck, Germany). The deboned flesh was washed (1 part flesh:20 parts water) for 10 min. The washed flesh was packaged in plastic bags (1 Kg) and frozen at -30°F until used.

### Patty preparation

The minced flesh was thawed at 35°F for 18-20 hours prior to use. The minced flesh was mixed (Univex Mixer, Model 1222) for 10 min with the percentages of NaCl, TPP and NaAlg. as indicated by the experimental design. The patties were then weighed (90 gm per patty) and shaped with a Petrie dish. Each patty was dipped in a 0.50% CaCl<sub>2</sub> solution, drained, battered and breaded, then deep fat fried for 30 sec at 400°F. The patties were cooled, placed in coded bags and frozen at 0°F until evaluation by objective and sensory methods. On the day of evaluation the patties were cooked for 30 min at 400°F.

A schematic diagram indicating the procedure is shown in Figure 1.

### Objective analyses

Textural properties were evaluated in terms of breaking force (BF). The appropriate attachment (plunger #13, 1.9 cm diameter) was fitted on an Instron Universal Testing Machine, Type TM 1900 (Canton, MA) using cell CCTM, chart speed 5 cm/min, crosshead speed 2 cm/min and clearance

from load cell 2 mm. Breaking force by compression was evaluated as the first peak height of the force curve, which is the amount of force required for the plunger to break the specimen. Two replicate patties were used for each treatment with three measurements on each patty.

The apparent viscosity for the various combinations of the minced mullet flesh was determined. A Brookfield Syncro-Lectric Viscometer (Model RVT) equipped with spindle #4 was used for viscosity measurements. A water bath was maintained at  $4 \pm 1^\circ\text{C}$ . Dial readings were recorded after 60 sec at 0.5, 1.0, 2.5, 5.0, 10, 20 and 100 RPM. Apparent viscosity was calculated from the dial readings.

### Sensory evaluation

In the sensory evaluation study, nine experienced panelists were presented with four samples and asked to determine if they were firmer/softer than the reference (Ref). The Ref was one of the treatment combinations. The data was evaluated using a nine-point rating scale where 9 = extremely firmer than Ref, 1 = extremely softer than Ref and 5 = similar to Ref.

### Statistical design and analysis

A Response Surface Analysis (RSA) (2) was performed on the data. Three factors (NaCl, TPP and NaAlg.) were chosen for their effect on the various responses and the function was expressed in terms of a quadratic polynomial equation which measures the linear effect, quadratic effect and interaction effect. Contours of a constant value were calculated by fixing one factor at a constant value and solving the equation for combinations of the other two factors. This type of statistical analysis enables one to predict responses and observe trends for combinations of the factors not necessarily included in the actual experiment.

Five levels of each of the three factors (Table 1) were chosen for study. Seventeen combinations were tested in random order according to a central composite design for three factors. The surface contours were calculated by computer and the data were analyzed using the SAS program package for analysis of variance. The levels studied were 0 to 1.0% NaCl, 0 to 0.50% TPP and 0.10 to 1.0% NaAlg.

## RESULTS AND DISCUSSION

### Statistical analyses

The coefficient of determination or  $R^2$  value is a measure of how well the empirical model fits the actual data. The closer the  $R^2$  is to one, the better the empirical model fits the actual data (9). The smaller the value of  $R^2$ , the less influence the independent variables in the model have in explaining the behavior variation. The  $R^2$  for breaking force was low (0.55); therefore, only general trends were considered. The  $R^2$  for the sensory evaluation of texture was high (0.94); therefore predictions concerning this response were made with confidence.

Coefficient estimates for two regression models and the results of significance tests on the coefficients are shown in Table 2. According to the significance test on estimates, the NaAlg. is probably the

primary factor influencing texture (sensory) since  $\beta_3$  is highly significant. No significant factors were indicated for the breaking force, which was not surprising due to the low  $R^2$ .

#### Breaking force

Due to the low  $R^2$  obtained for the breaking force of the mullet patties, only trend analysis was considered (Figures 2a-2c). The trend indicates as the NaAlg. concentration was increased, the contours shifted upward and the BF was decreased. At a fixed NaAlg. level, if the TPP level was decreased to some minimum value, then the NaCl concentration must be decreased in order to maintain the same force. As the TPP was increased from this minimum point it was necessary to increase the NaCl concentration in order to maintain the same force. The minimum point for TPP was not within the experimental range for all force values at each NaAlg. level. As the NaAlg. level was increased, the minimum for TPP was increased. At any NaAlg. level, the contours appeared to be symmetric around the horizontal axis so that the minimum TPP level for each force value was obtained at the same NaCl concentration. The concentration of NaCl that was the "transition point" increased (contours shifted toward right) as the NaAlg. concentration was increased. At a constant TPP level, as the NaCl concentration was increased from 0% to this "transition point," then the BF was increased; as the NaCl was increased from this point then the BF decreased. At a constant NaCl level, as the TPP concentration was increased, then the BF increased. A softer patty was obtained at higher NaCl-TPP-NaAlg. levels.

#### Apparent viscosity

The apparent viscosity for the seventeen uncooked formulations was determined. The flow behavior index,  $n$  is an indication of the type of flow behavior. If  $n = 1$  then the product is Newtonian, which means the viscosity remains constant over a range of shear rates. If  $n < 1$ , then the behavior is pseudoplastic or the apparent viscosity decreases as the shear rate increases. If  $n > 1$ , this indicates dilatant behavior or an increase in apparent viscosity with an increase in shear rate. Generally, the samples indicated pseudoplastic behavior. Figure 3 is an example of one such curve.

If a RSA was run for the apparent viscosity of the samples at a given shear rate this would be of value to processors using an extruder for patty formulation. If they know which combination is acceptable then they would be able to determine which shear rate would give the best viscosity measurement for extrusion.

#### Sensory evaluation

The  $R^2$  for the sensory evaluation of texture was high so predictions concerning optimum response may be made with confidence. Panelists were asked to compare the samples with a Ref patty and determine if the texture was softer/firmer than the Ref. Generally, as the NaAlg. was increased, the contours shifted upward and toward the right of the central axis (Figure 4a-4c). At NaAlg. levels up to 0.55% patties both

softer and firmer (3.5 to 6.2) than the Ref (5.0) were found. For most responses, as the concentration of NaCl was increased then it was necessary to decrease the TPP in order to obtain the same response. Some contours firmer than or equal to Ref became curvilinear in character so that two concentrations of NaCl would produce the same response at a constant TPP. Also either an increase in NaCl or TPP caused an increase in firmness. As the NaAlg. concentration increased beyond 0.55%, only patties softer than the Ref were obtained; if the TPP was increased above 0.25%, then the firmness decreased.

## CONCLUSIONS

A correlation between the sensory evaluation and the breaking force was not made since the  $R^2$  was low for the breaking force; however, it would be possible to use the sensory evaluation to predict the optimum response.

Some general conclusions are as follows: 1) the patty formulation exhibit a pseudoplastic flow behavior, 2) regression analysis indicated that the effect of NaAlg. was highly significant ( $\alpha = .01$ ) for sensory evaluation, 3) the firmness of the patties decreases as the NaAlg. level increases, 4) according to sensory evaluation, a high TPP level will produce firmer patties at low NaAlg. levels; however, at high NaAlg. levels, a low TPP level will produce the firmer patties, 5) generally, panelists indicated the greater the salt concentration (TPP and NaAlg. constant) the firmer the patty.

TABLE 1

RANGES OF INDEPENDENT VARIABLES USED IN PATTY FORMULATION

NaCl	0 - 1.0%
TPP	0 - 0.5%
NaAlg.	0.10 - 1.0%

TABLE 2

REGRESSION COEFFICIENTS FOR TEXTURE RESPONSE  
OF MULLET PATTIES

	<u>BREAKING FORCE</u>	<u>SENSORY EVALUATION</u>
$\beta_0$	1237.992	4.875**
$\beta_1$	20.019	0.272*
$\beta_2$	68.301	0.221
$\beta_3$	-159.174	-0.695**
$\beta_{11}$	-150.609	-0.104
$\beta_{22}$	1.087	-0.125
$\beta_{33}$	-41.329	-0.060
$\beta_{12}$	1.875	-0.311*
$\beta_{13}$	14.375	-0.031
$\beta_{23}$	-17.917	-0.249

\* = significant at  $\alpha = .05$ \*\* = significant at  $\alpha = .01$ 

1 = NaCl; 2 = TPP; 3 = NaAlg

## PATTY PREPARATION

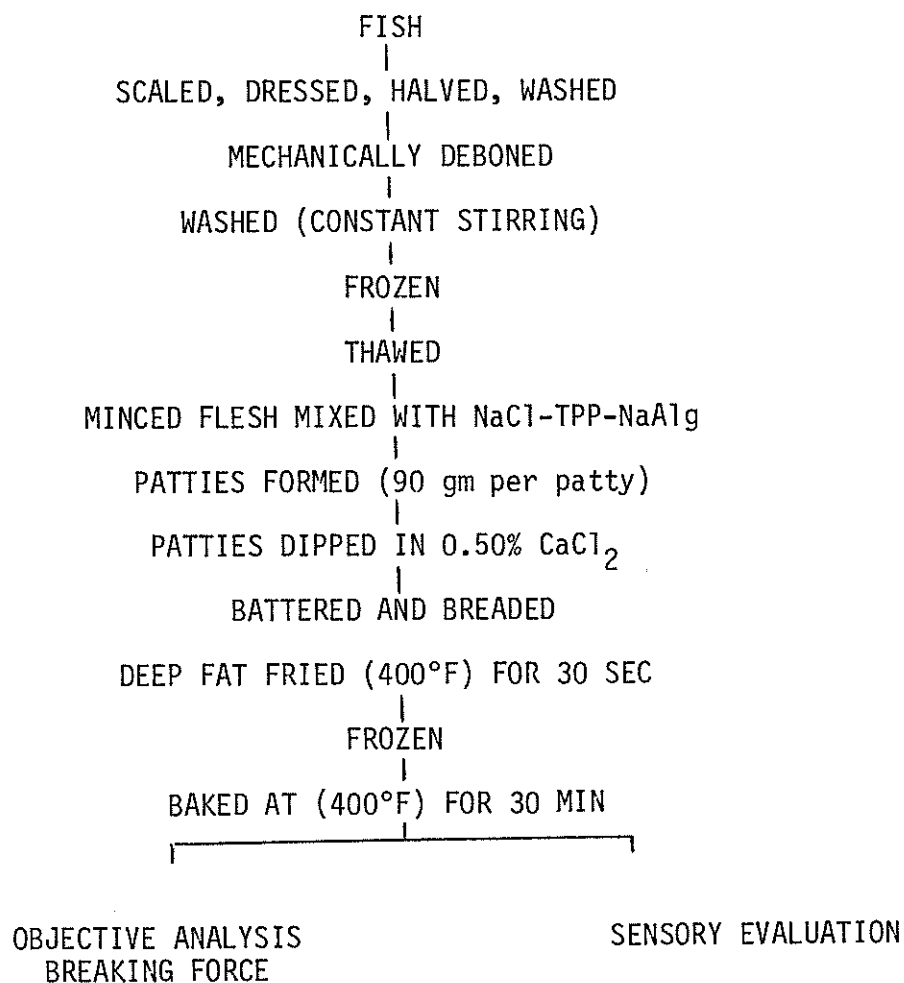


FIGURE 1. Flow chart for preparation of minced fish patties.



Alginate = 0.10 %

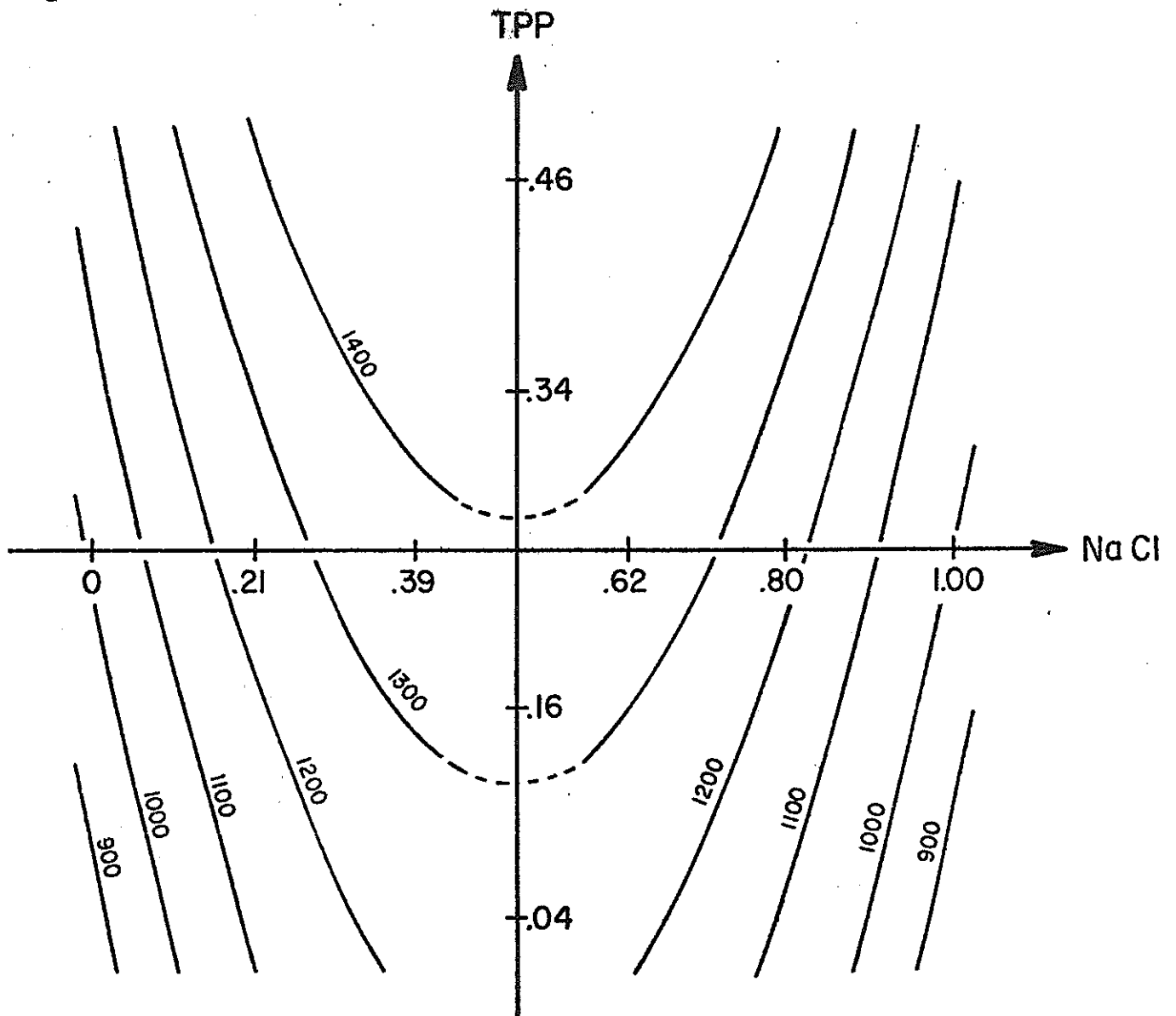


FIGURE 2a. Response surface contours for TPP and NaCl effect on texture quality response (break force) of minced mullet at 0.10% alginate level.

Alginate = 0.415 %

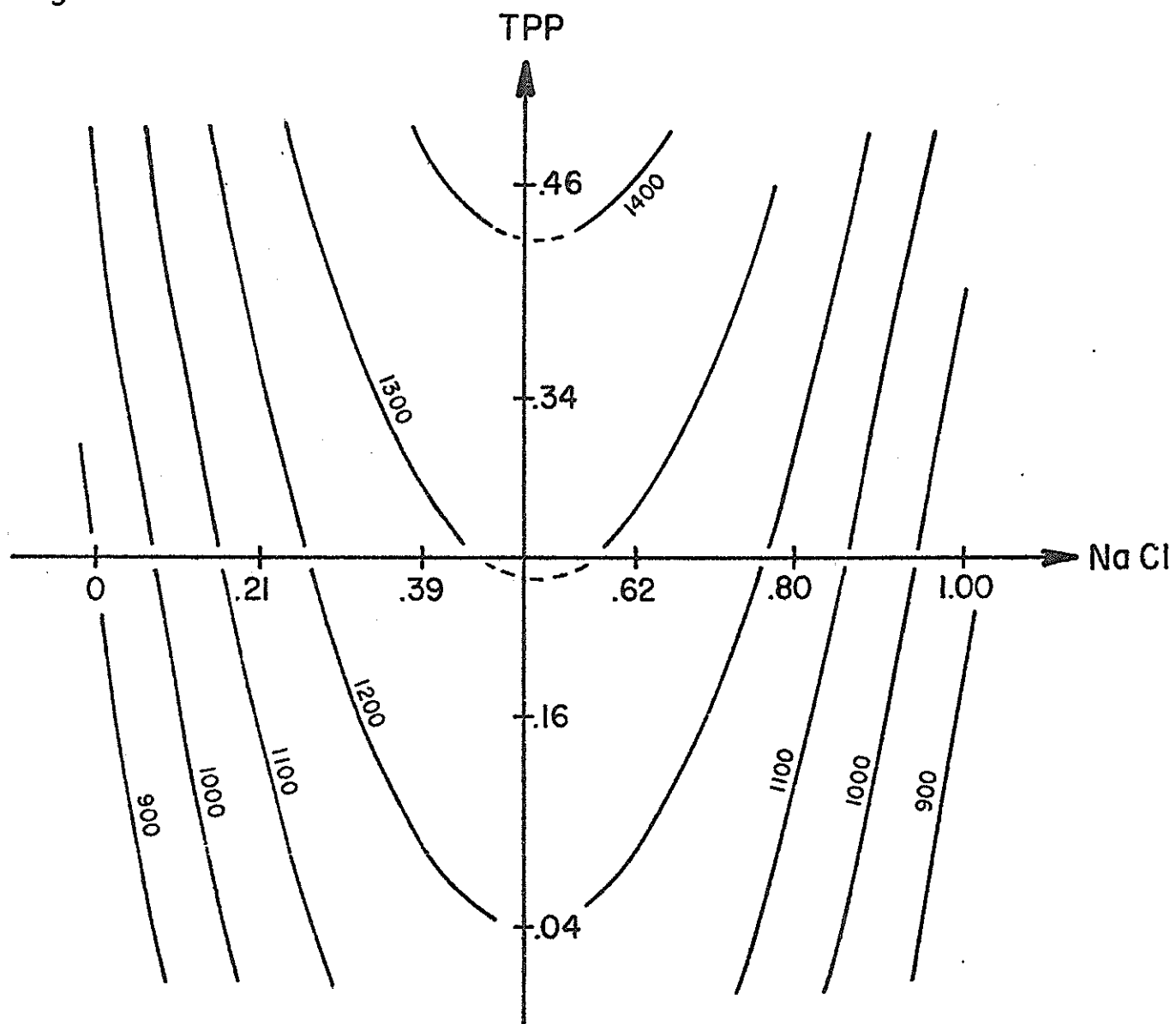


FIGURE 2b. Response surface contours for TPP and NaCl effect on texture quality response (breaking force) of minced mullet at 0.415% alginate level.

Alginate = 0.685 %

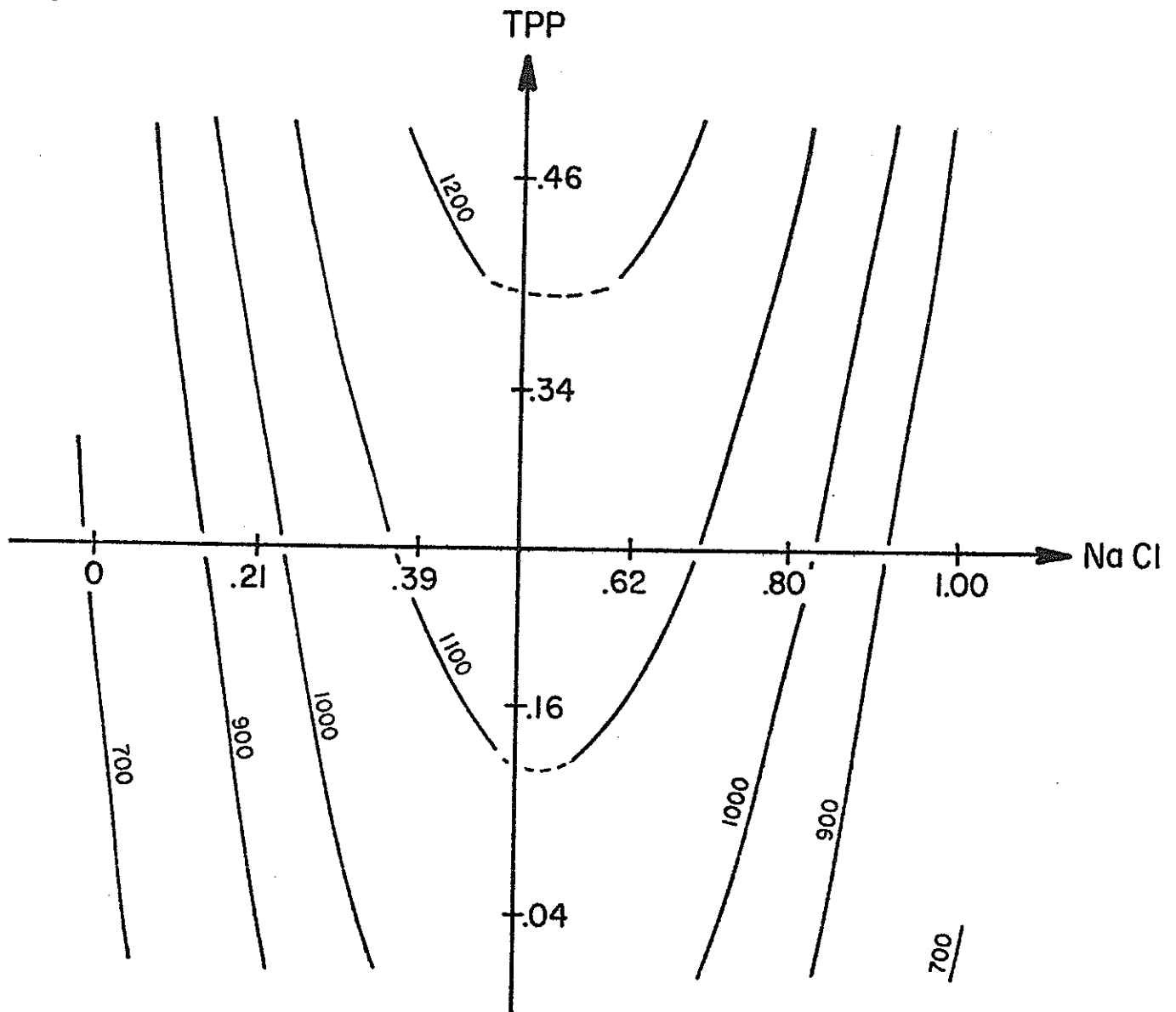


FIGURE 2c. Response surface contours for TPP and NaCl effect on texture quality response (breaking force) of minced mullet at 0.685% alginate level.

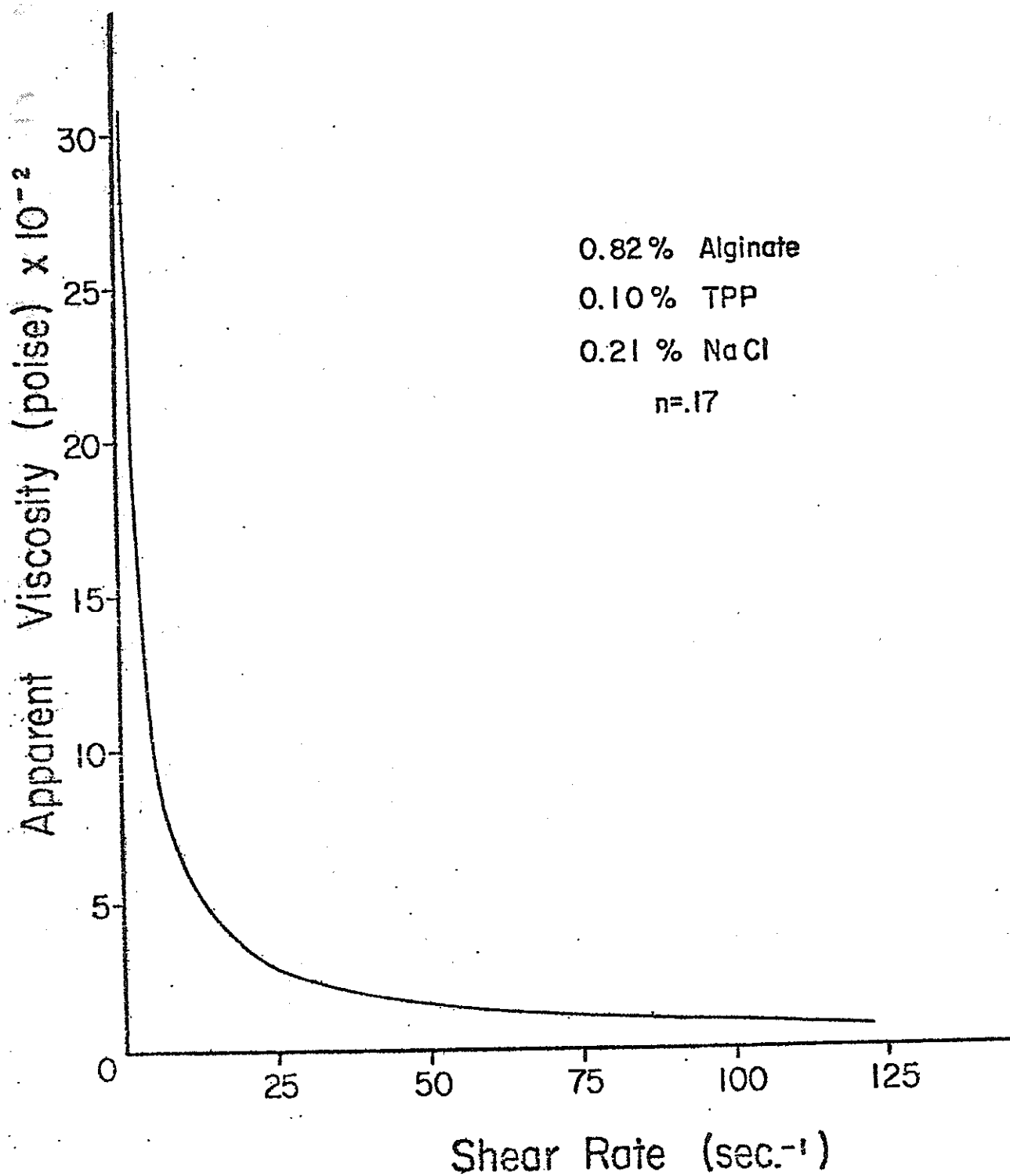


FIGURE 3. Effect of shear rate on the apparent viscosity of minced mullet at 0.82% alginate, 0.10% TPP and 0.21% NaCl.

Alginate = 0.10%

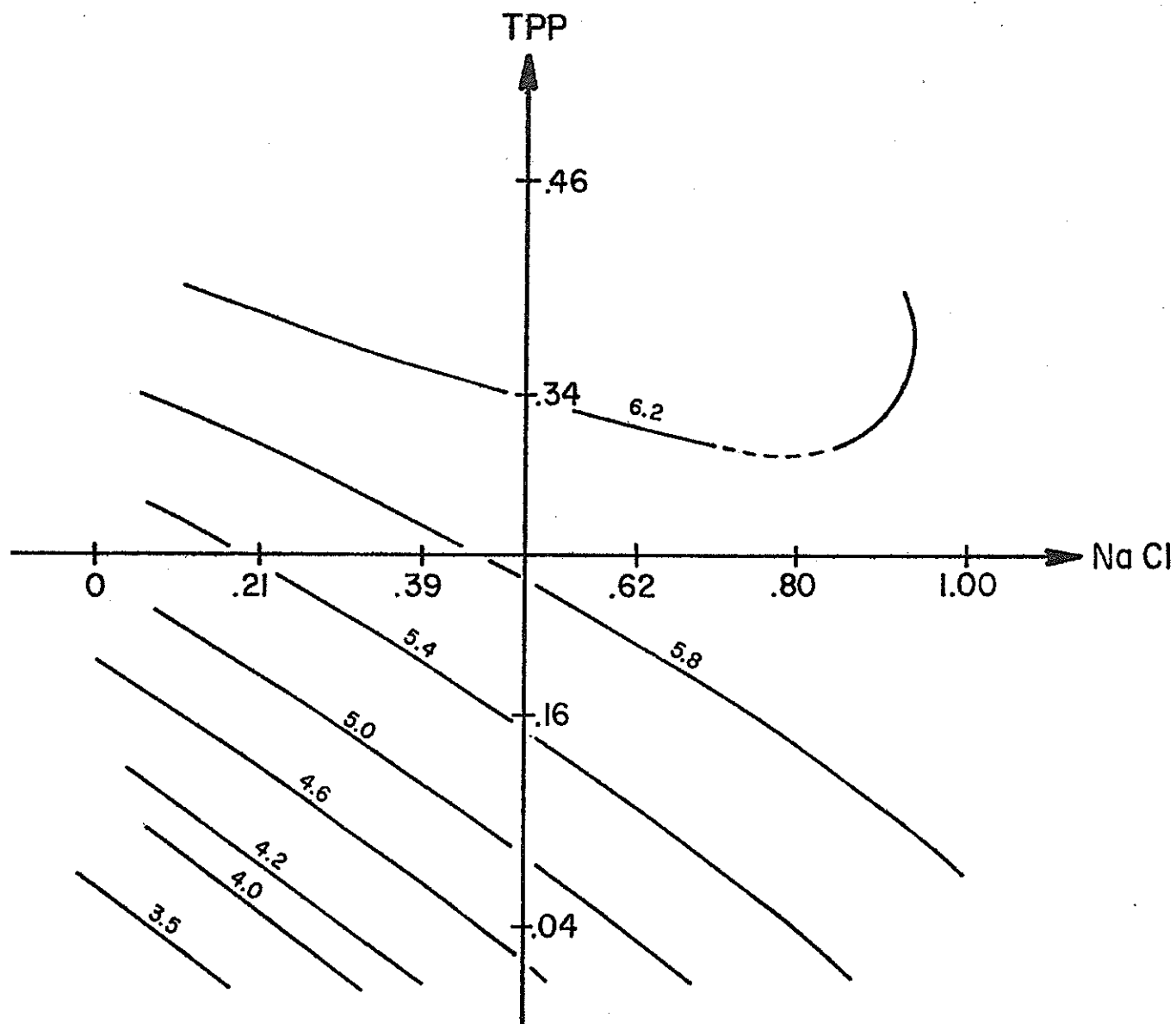


FIGURE 4a. Mixture response surface contours for TPP and NaCl effect on texture sensory quality response of minced mullet at 0.10% alginate level.

Alginate = 0.415%

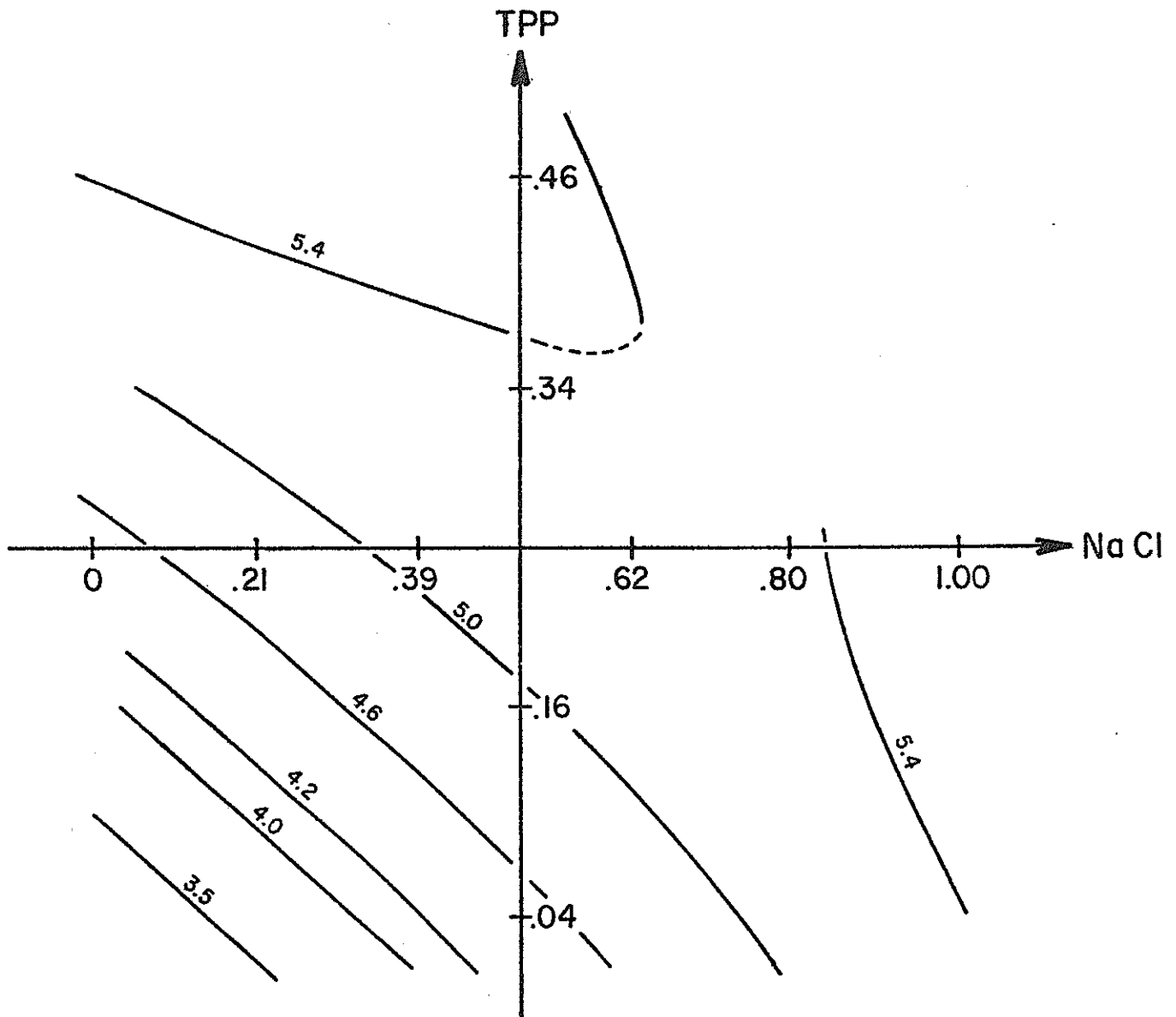


FIGURE 4b. Response surface contours for TPP and NaCl effect on texture sensory quality response of minced mullet at 0.415% alginate level.

Alginate = 0.685 %

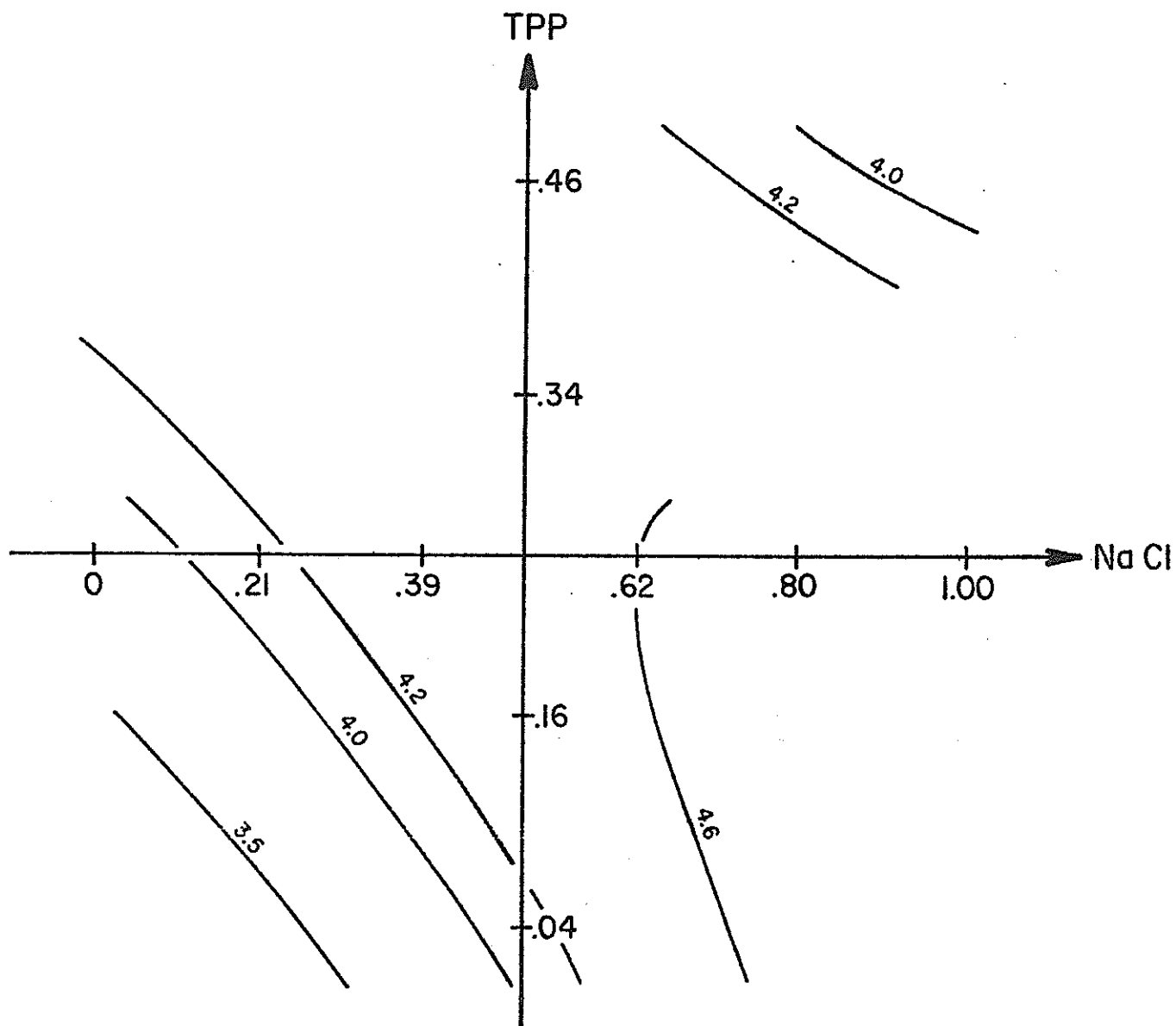


FIGURE 4c. Response surface contours for TPP and NaCl effect on texture sensory quality response of minced mullet at 0.685% alginate level.

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# THE SPOILAGE MECHANISM OF GULF OF MEXICO FINFISH STORED IN A MODIFIED CO<sub>2</sub> ATMOSPHERE

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## INTRODUCTION

Traditionally, fresh fish have been preserved and marketed by placing them on ice. However, the concept of packaging fresh fish offers an interesting alternative which can protect the fish from contamination, provide easier handling and present a more attractive product, therefore promoting marketing potential (Hardy and Hobbs, 1968; Huss, 1971).

Controlled atmosphere packaging is currently being used to prolong the shelf-life of fresh poultry and meats such as pork and beef. This type of packaging is used in the meat industry to suppress the growth of common spoilage organisms where at the same time oxygen tension is kept high enough to provide a desirable bright red meat color resulting from oxygenation of fresh meat pigments (Bala et al., 1977; Taylor and MacDougall, 1973; Baran et al., 1969; Silliker et al., 1977).

Since fish is food commodity that spoils relatively quickly, controlled atmosphere packaging offers a new idea for prolonging the shelf-life of fresh fish. This research is part of a project that is presently investigating the processing and marketing of fresh fish from the Gulf of Mexico, which have been put into oxygen-permeable overwrapped retail tray packs and placed into CO<sub>2</sub> oxygen-impermeable backflushed master cartons. The study is concerned with evaluating the differences in the biochemical and microbiological spoilage mechanisms of fresh fish packaged in a CO<sub>2</sub> atmosphere as compared to the present method of holding fresh fish on ice.

## MATERIALS AND METHODS

### Preparation and Storage of Samples

The two types of Gulf of Mexico finfish that were used were the speckled trout (Cynoscion nebulosus) and golden croaker (Micropogon undulatus.) Both are found to be fairly abundant in the Gulf of Mexico and are considered to be difficult to maintain. These fish were processed (gilled and gutted), placed on retail tray packs and

overwrapped. The packages were placed into a CO<sub>2</sub> backflushed master carton, sealed and covered with ice. The cartons were picked up and transported to the Seafood Laboratory in College Station.

For each species of fish, a total of six master cartons, each containing eight individual packages of fish, were used for testing purposes. Four master cartons designated as BF<sub>1</sub>, BF<sub>2</sub>, BF<sub>3</sub> and BF<sub>4</sub> were backflushed with CO<sub>2</sub> and stored at 4°C. Every two days one master carton was broken and tested. The remaining packages of fish in the master carton were placed in a retail case and tested each day.

Two master cartons designated NBF<sub>1</sub> and NBF<sub>2</sub> which were not backflushed with CO<sub>2</sub> served as controls. One master carton (NBF<sub>1</sub>) was stored at 4°C and a sample was removed and tested each day that a master carton that had been backflushed with CO<sub>2</sub> was broken. Packages from the second master carton (NBF<sub>2</sub>) were stored in the retail case from day 0 and tested every day.

### Microbiological Analyses

All samples were analyzed for total number of bacterial per unit of surface area. The skin samples (10 cm<sup>2</sup>) were obtained by placing a sterile template on the surface of the package and removing a portion of skin and package film with a sterile scalpel and sterile tweezers. The skin sample and packaging material were then placed in a dilution bottle containing 100 ml of sterile 0.1% peptone with sterile glass beads. The bottle was closed tightly and shaken rapidly up and down 30 times. Total bacteria numbers were determined by spread plating on Standard Methods Agar (BBL) appropriate aliquots from the dilution bottles containing the known portion of aseptically removed fish skin and 0.1% peptone. Plates were counted after 48 hours at 25°C.

### Chemical Analyses

For chemical analyses, 50 grams of fish were placed in a Waring blender with 100 ml (1:2 ratio) of 7% trichloroacetic acid solution and blended. The mixture was filtered to remove the protein precipitate. Total volatile nitrogen was determined by the modified Conway microdiffusion method (Obrink, 1955). The procedure utilizes saturated Na<sub>3</sub>PO<sub>4</sub>/KOH as the releasing agent (Cobb et al., 1973), 3.1% H<sub>3</sub>BO<sub>3</sub> with 1 ml indicator (Conway, 1958) added to 50 ml solution and 0.02 N H<sub>2</sub>SO<sub>4</sub> as the titrating agent. Diffusion time was 2 hours before titrating with a Metrohm Herisau Dosimat (Brinkman Instruments). Total volatile nitrogen analyses were expressed as mg Nitrogen/100 g fish (mg N/100 g fish).

## RESULTS AND DISCUSSION

Figures 1 and 2 express the relationship of total numbers of bacteria/cm<sup>2</sup> and days in storage of trout and croaker. Initial aerobic plate counts found on trout (DAY 1) and croaker (DAY 0) were  $1.6 \times 10^5/\text{cm}^2$  and  $7.2 \times 10^4/\text{cm}^2$  respectively. These fish which were

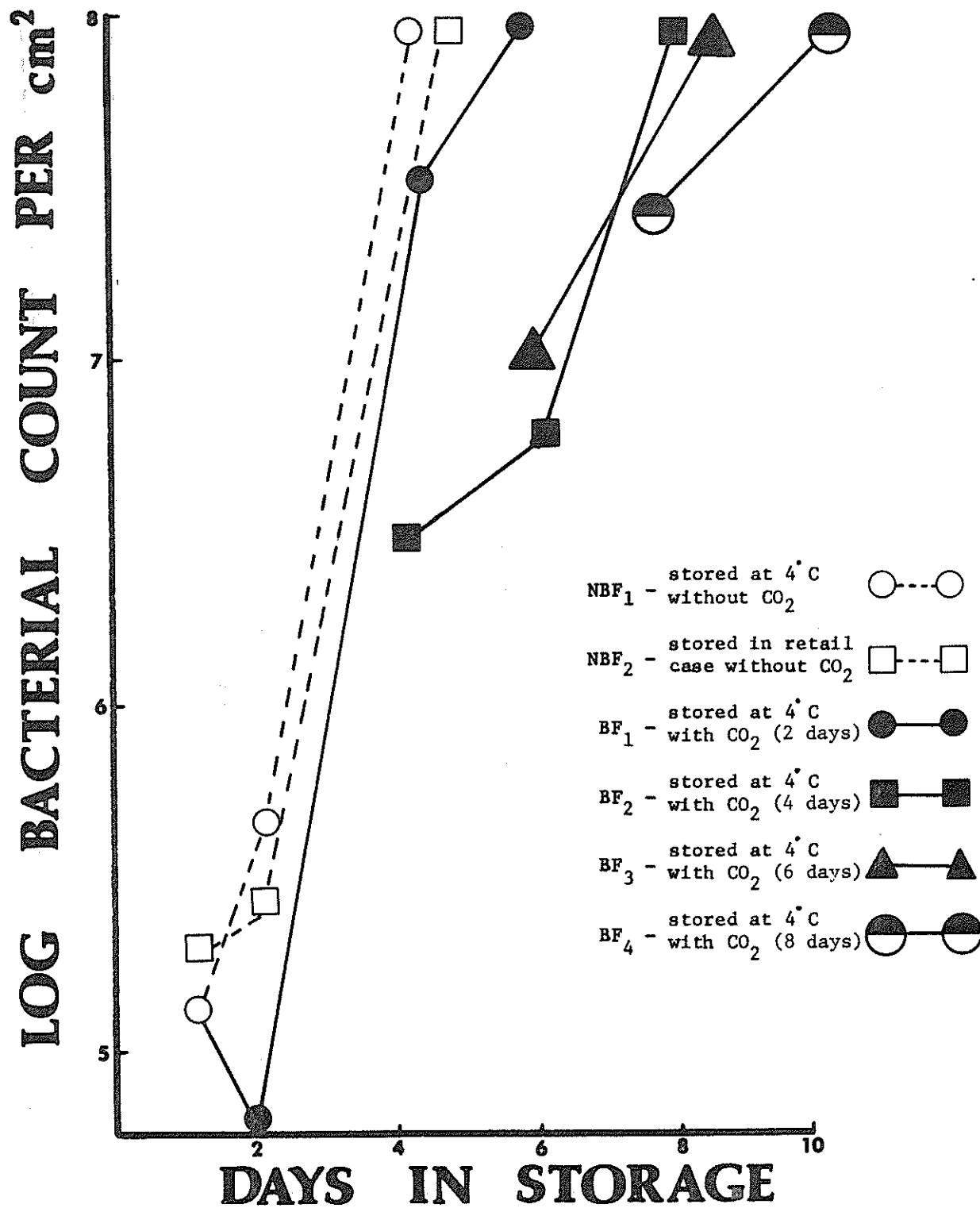


FIGURE 1. Total aerobic plate counts of trout stored at 4°C and in a retail case (0-10°C) packaged with and without CO<sub>2</sub>. Initial points show day of removal from CO<sub>2</sub> master carton.

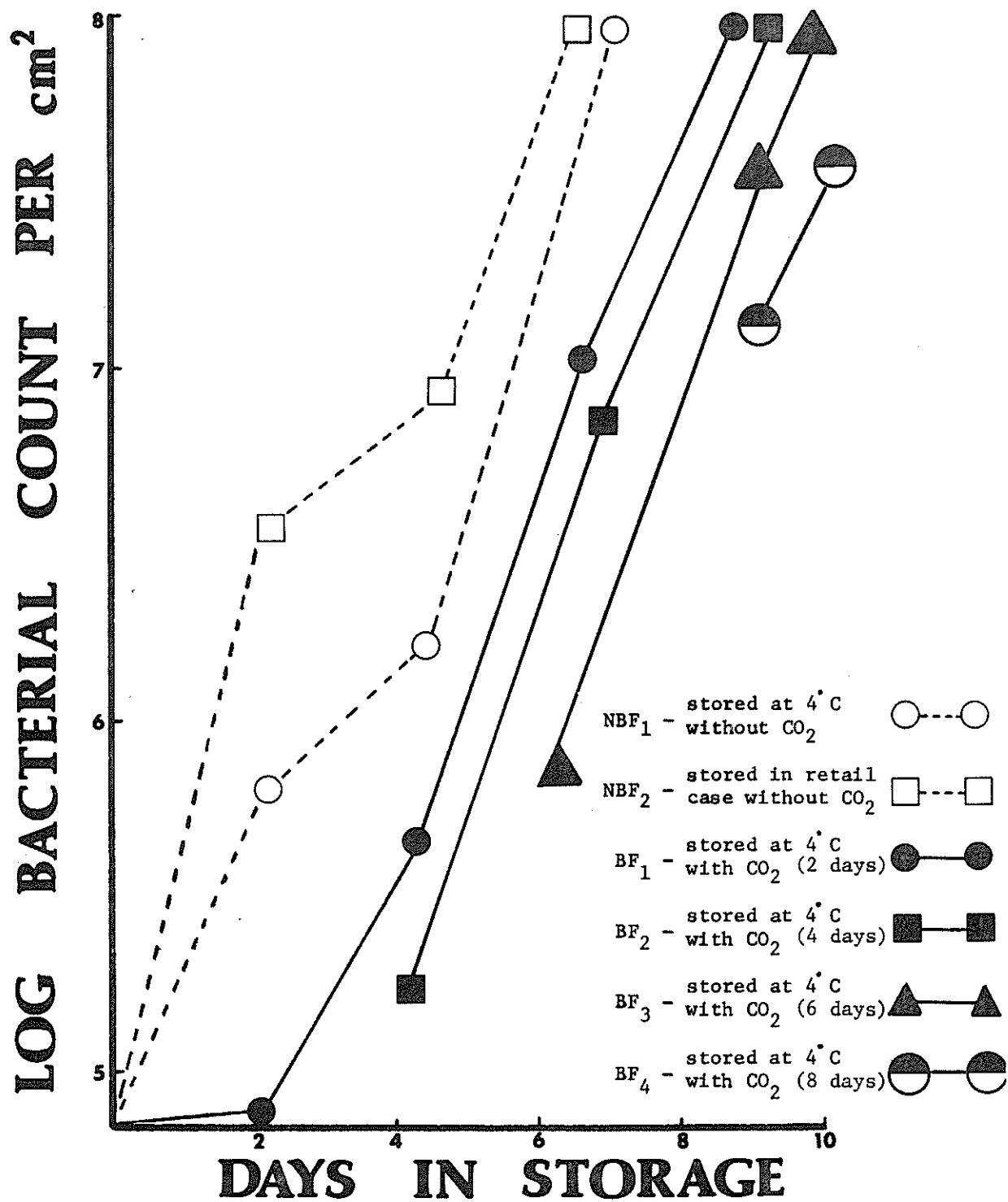


FIGURE 2. Total aerobic plact counts of croaker stored at 4°C and in a retail case (0-10°C) packaged with and without CO<sub>2</sub>. Initial points show day of removal from CO<sub>2</sub> master carton.

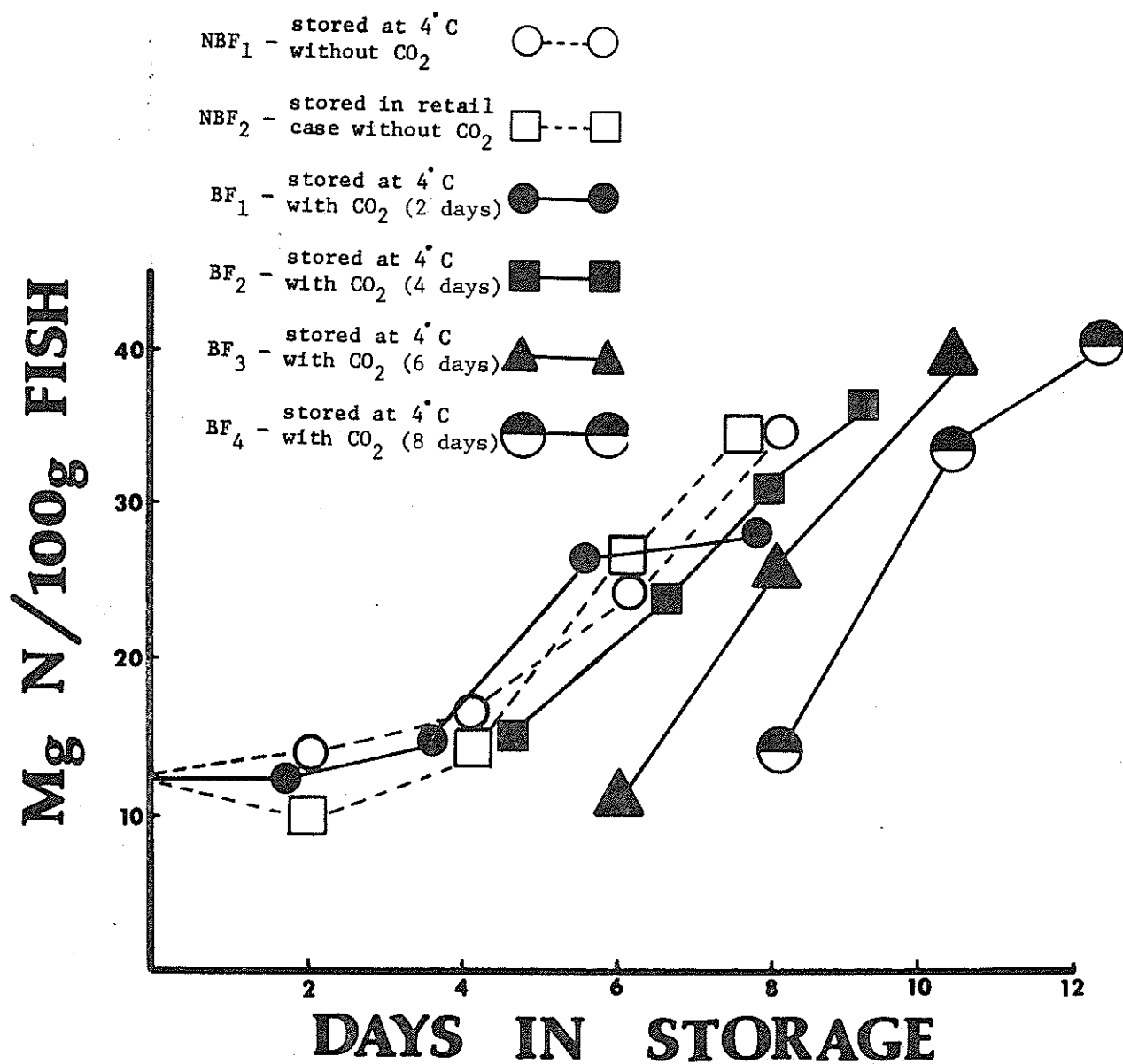


FIGURE 3. Total volatile nitrogen of trout stored at 4°C and in a retail case (0-10°C) packaged with and without CO<sub>2</sub>. Initial points show day of removal from CO<sub>2</sub> master carton.

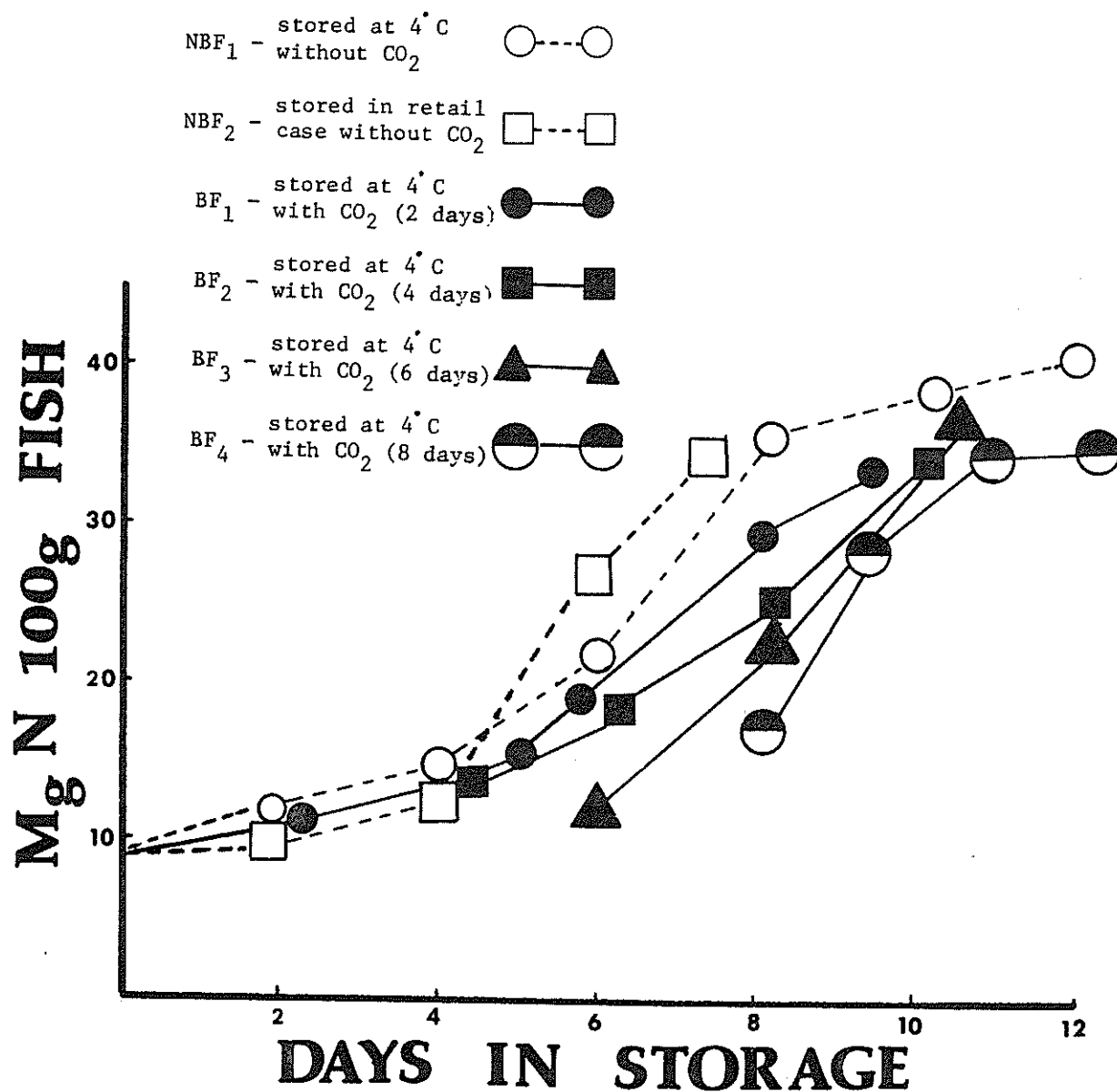


FIGURE 4. Total volatile nitrogen of croaker stored at 4°C and in a retail case (0-10°C) packaged with and without CO<sub>2</sub>. Initial points show day of removal from CO<sub>2</sub> master carton.

placed in master cartons backflushed with CO<sub>2</sub> had significantly lower counts than fish stored without CO<sub>2</sub>. At two, four and six days, there is almost a complete log difference in populations of bacteria between the controls (NBF<sub>1</sub> and NBF<sub>2</sub>) and the packaged fish stored in CO<sub>2</sub> (BF<sub>1</sub>, BF<sub>2</sub> and BF<sub>3</sub>). This finding is consistent with earlier studies that showed there is a marked inhibition of microbial growth by carbon dioxide on pork and beef (Huffman 1974; Silliker et al., 1977). Once the fish were taken out of a controlled atmosphere containing CO<sub>2</sub> and placed in a retail case, a rapid rise in bacterial growth could be seen.

The presence of CO<sub>2</sub> not only limits microbial growth but also causes changes in the type distribution of microorganisms. When stored unpackaged in air, the microbiological population on the fish changed from mainly gram-positive (coryneforms and Micrococcus) to gram-negative (Pseudomonas and Moraxella). When stored under a CO<sub>2</sub> atmosphere the fish showed the opposite distribution in bacterial types. With CO<sub>2</sub> there was an increase in gram-positive organisms, predominantly Lactobacillus. When fish were removed from a CO<sub>2</sub> atmosphere and put back on ice, this trend was immediately reversed. The inhibition that CO<sub>2</sub> caused on bacterial numbers also terminated when the fish was removed from this atmosphere as an immediate acceleration in the outgrowth of gram-negative organisms was observed.

Figures 3 and 4 express mg N/100 g of fish produced during days of storage in trout and croaker. Initial mg's of total volatile nitrogen produced at DAY 1 in trout and DAY 0 for croaker were 13.7 mg N/100 g and 9.5 mg N/100 g, respectively. Fish which were in a controlled atmosphere containing CO<sub>2</sub> had substantial lower amounts of volatile nitrogen produced than fish stored without CO<sub>2</sub>. At six and eight days, there is a vast difference in mg N/100 g of fish between the controls (NBF<sub>1</sub> and NBF<sub>2</sub>) and fish packaged in CO<sub>2</sub> master cartons (BF<sub>3</sub> and BF<sub>4</sub>). It has been quoted in literature (Montgomery et al., 1970; Farber, 1965) that 30 mg N/100 g of fish is a general indication of spoiled before this point was reached.

#### CONCLUSION

In summary, an important point to remember is that if the initial quality of the fish is not good, control atmosphere packaging with carbon dioxide has no effect. However, if a good quality of fish is packaged in CO<sub>2</sub>, there is a significant effect in retarding the growth of bacteria, changing the microbial species distribution, and the amount of total volatile nitrogen produced during storage, implicating a possible extension of shelf-life of 2-4 days.

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POSSIBLE HAZARDS FROM USE OF CHLORINE  
BY THE FOOD INDUSTRY

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Chlorine is a Generally Recognized As Safe listed chemical. The FDA Good Manufacturing Practice regulation 21 CFR S110 lists the use of chlorine as a sanitizing agent and indicates that the chlorination process is effective in destroying vegetative cells of pathogenic and spoilage bacteria, as well as other microorganisms. Initially, it was thought that chlorine reacted with organic materials to produce innocuous inorganic and organic compounds. Recent studies, however, using radioactive chlorine have estimated that from .5 to 3.1% of the chlorine used for sanitizing purposes ends up as chlorinated organic compounds, some of which have been shown to be hazardous. Questions concerning the use of chlorine compounds as direct and indirect food additives have arisen because of the potential hazards associated with these compounds.

This afternoon I hope to convey to you the impression that the Food Scientists must be a concerned group of investigative scientists, who are interested in identifying and quantifying potential hazards that may arise from the use of chlorine as a direct or indirect additive in foods. It is not our intent to indicate that the use of chlorine by the food industry is a problem. Rather that there is concern that a potential problem may exist.

At the present time, over 10.5 million tons of chlorine are produced annually in North America. Of this figure, only 3 to 4% is used for sanitation purposes including waste treatment, potable water, household uses, and the food industry.

Chlorine is a GRAS listed chemical. The FDA Good Manufacturing Practices regulation 21 CFR S110 lists the use of chlorine as a sanitizing agent and indicates that the chlorination process is effective in destroying vegetative cells of pathogenic bacteria, as well as other microorganisms. In the past, the food industry has used chlorine to remove off-odors, bad tastes, bleaching of color, prevention of biofouling of cooling systems, and as the principle sanitizer and bactericide.

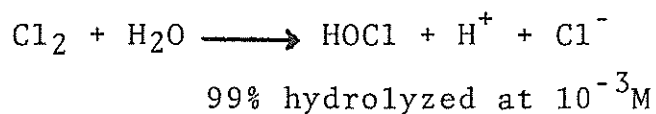
Of the over 250 compounds that have been identified in the nations drinking water, 34 have been identified as halogenated.

Health hazards associated with chlorinated inorganic compounds are considered to be very minimal because they are short-lived and can be destroyed by treatment with reducing agents, such as sulfur dioxide or sodium thio-sulfate. Chlororganic compounds in water systems are not short-lived and are not destroyed by reduction reactions. Chloroform is the most commonly recognized reaction product and may be present at concentrations as high as 100 ppb. The remaining products are the result of reactions with nitrogenous bases and aromatic or aliphatic compounds. They are generally present at concentrations less than 50 ppb and are nonvolatile. The principal problems with these compounds is that in addition to not being short-lived, or readily destroyed by reducing agents, they can be readily substituted into biological compounds as a substitute for the parent molecule. It is also important to consider that the substitution or addition of chlorine into molecules can occur in second generation reaction products, which may result in products that are a greater health hazard than the parent compound. Organic nitrogenous compounds including the pyrimidine bases, cytosine and uracil, form chloro analogs when reacted with hypochlorous acid (Kopperman et al., 1975). Mutagenicity studies involving chlorinated genetic base materials indicates that 5-chlorouracil was readily incorporated into the DNA of mice who were exposed to the chlorinated nitrogenous base in their drinking water (Cumming, 1975). Specific locus mutation studies performed on mice indicate that at the levels currently found in water supplies, these chlorinated bases cannot be shown to be a significant mutagenic or carcinogenic hazard to humans at the present time. Data are available, however, which shows that chlorinated compounds can readily accumulate in biological tissues (Cumming, 1975 and Walton and Cumming, 1976).

Specific instances where the use of chlorine in food processing may be of concern because of the direct contact of high levels of chlorine or hypochlorous acid with foods. Examples of such instances would be the spraying of beef carcasses with 200-300 ppm available chlorine during hydro-cooling of the freshly slaughtered steers, cooling of poultry in rocker chillers using ~20 ppm available chlorine solutions following evisceration, and the addition of 1 to 6.6 ppm chlorine dioxide to flour for the purpose of bleaching the flour. In the first two instances, although the level of available chlorine is a few hundred ppm, evaporation will result in higher levels on the surface of the product. Studies carried out by two Canadian scientists, Cunningham and Lawrence (1977), have shown that red meats

The sanitation benefits derived from the use of chlorine and other halogens by the food industry are obvious and undisputed. It was assumed that in performing this function, chlorine reacted with organic materials to produce  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{Cl}_2$  and innocuous inorganic and organic compounds. However recent studies using  $^{36}\text{Cl}$  chlorine have estimated that from 0.5 to 3.1% of the 400,000 tons of chlorine used for sanitation purposes ends up as chlorinated organic compounds, some of which have been shown to be a health hazard. Thus, questions have arisen concerning the use of chlorine in drinking water, treatment of waste effluents, and as a sanitizing agent in the food industry.

Questions concerning the use of chlorine, hypochlorous acids and chlorine bleaching compounds as direct and indirect food additives are because of the reactivity of these chlorine compounds. Elemental chlorine ( $\text{Cl}_2$ ) is almost totally hydrolyzed in water to form hypochlorous acid and a free chloride ion.



Hypochlorous acid, a weak acid having a  $\text{pK}_a$  of 7.5, is the most-active form of chlorine as a sanitizer. Although other transient species of chlorine are present as a result of the hydrolysis reaction, including hypochlorous hydronium ions, chloronium ions and the  $\text{Cl}^+$  species, some of which may be more reactive than hypochlorous acid, the  $\text{HOCl}$  still shows the greatest net relative reactivity because of its specific reactivity and the large fraction of the total chlorine present as this species. The concern that hypochlorous acid could represent a possible hazard when used as a direct or indirect food additive is not indiscriminate, but rather is based on well-defined reaction mechanisms and pathways involving chlorine and organic compounds. Electrophilic substitution and addition reactions involving the incorporation of hypochlorous acid into nitrogenous bases, aromatic ring compounds and unsaturated aliphatic compounds have been described in aqueous solutions including chlorination of water containing organic matter by various researchers, including Morris (1967 and 1977), Rook (1975 and 1974), Bellar et.al. (1974), and Jolley (1975 and 1976).

Although little research has been carried out involving aqueous chlorination reactions in food systems, considerable data from studies done with drinking water, waste water and sewage effluents are available.

and poultry contained various levels of chlorine after immersion for 2 to 24 h in a 200 ppm solution of <sup>36</sup>chlorine, however, toxicity studies were not carried out.

Studies by Cunningham and Lawrence (1976 and 1977) involving the feeding of chlorinated gluten flour, flour lipids, corn oil, and unsaturated fatty acids to rats showed decreased growth rate and increased liver size in the test animals. Whether the presence of chlororganics in foods is harmful to man at level which may be present with current processing procedures, no one can say. Nor can it be said that they are not a hazard. What can be said is that there is a need for further research and for the use of chlorine in a safe, efficacious manner.

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## A REVIEW OF THE LOUISIANA CHOLERA OUTBREAK

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### INTRODUCTION

Cholera (a foodborne disease caused by the bacterium Vibrio cholerae) was responsible for several disease epidemics in Louisiana before the 1978 outbreak. For example, in 1832-33, over 6,000 people in New Orleans alone died from this disease. This was one of the bleakest years in New Orleans' history. The population was 55,000, and more than one-seventh of the population died from yellow fever, cholera, and other diseases in a one-year period(1). Over the next 40 years, several epidemics of cholera afflicted the population with each succeeding epidemic tending to be less virulent than the previous ones. The dual honors of yellow fever and cholera were the impetus behind the formation of the State Board of Health by the Louisiana Legislature in 1855.

Widespread epidemics of cholera as described above are usually attributable to poor sanitation and improper water and sewage treatment. Modern sewage treatment plants, strict monitoring of municipal drinking water, and commercial food processing regulations have, for the most part, eliminated any possibility of widespread cholera outbreaks. There have been only two isolations of V. cholerae from humans in the United States in the past decade. One was taken from a man in Alabama and the other from a man in Texas (2). No cluster of indigenous cases of cholera had been reported in the country since 1911 (3).

### THE PROBLEM

On August 10, 1978, a 44-year-old man became ill with watery diarrhea, chills, fever and nausea. This patient was admitted to a hospital on August 13 as a result of dehydration. The hospital laboratory observed the strange occurrence of a pure culture of hemolytic colonies from a stool culture. An interested technologist continued to pursue the identification of the organism, and through biochemical reaction it was determined that the bacteria was a vibrio.

The culture was sent to the state laboratory where it was further identified as V. cholerae and forwarded to the National Center for Disease Control (CDC) for typing. The CDC confirmed the isolate as Vibrio cholerae 0-1, biotype El Tor, serotype Inaba. It is interesting to note, as reported in Morbidity and Mortality Weekly Report (MMWR), that the patient had recently eaten boiled shrimp and boiled crabs (2).

Health officials believed at the time that this was an isolated case and consequently did not expect additional cases. However, additional cases did begin to surface. The fifth suspected case was announced on September 25. CDC officials stated that all five persons had consumed boiled or steamed crabs within five days before becoming ill. It should be noted, however, that the crabs were harvested from different locations along 60 miles of the Louisiana Gulf Coast (5).

In the September 29 edition of MMWR, CDC officials announced that the United States would now be listed by the World Health Organization as having a cholera-infected area (4). According to this same report, an area is considered infected until 10 days have passed since the last case identified has died, recovered, or been isolated, and there is no epidemiological evidence of spread of that disease to any contiguous area.

By the end of September, 11 cases of cholera in Louisiana had been reported to CDC. Eight of the patients became ill and three patients were asymptomatic. Prior to becoming ill, all had eaten either steamed or boiled crabs. Those individuals with symptoms had eaten crabs within five days before onset of illness while the asymptomatic infected persons had eaten crabs within nine days before culture (5). Table 1 summarizes information on each patient as it relates to the cholera outbreak.

In early October, the Federal Food and Drug Administration (FDA) recommended to health officials in Louisiana that a broad area in coastal southwest Louisiana be closed to commercial and private crabbing. The failure to do so, said FDA, could result in seizure of crabs caught in those areas if they were introduced into interstate commerce. Louisiana health officials refused to follow the recommendations, stating that there was not sufficient evidence of live crabs being contaminated with V. cholerae. In addition, all water samples were negative for V. cholerae. Newspaper and radio reports indicated that on Friday, October 6, a shipment of Louisiana crabs was intercepted in Baltimore, Maryland, and destroyed at the urging of FDA as a precaution against the spread of cholera.

Shortly thereafter, a water sample taken from the Old Intra-coastal Waterway between the Schooner Bayou central structure and White Lake was confirmed as having cholera bacteria. Consequently, state health officials closed this specific area to commercial and private crabbing. Because of the uncertainty of further closing of crabbing waters and the posting of FDA inspectors at airports in Baton Rouge and New Orleans to examine and/or sample interstate

shipments of crabs, the industry representing the affected area petitioned the Louisiana Cooperative Extension Service for guidance

## APPROACH TO THE PROBLEM

### Sampling Program

Shortly after it was determined that the first few illnesses were not isolated cases, appropriate Federal, State, and local agencies began an intensive sampling program. As of March 8, 1979, 491 live crab samples involving approximately 2,455 crabs failed to yield any positive results; of 109 shrimp samples involving approximately 1,448 shrimp, one sample was positive; 75 raw oyster samples involving approximately 923 oysters failed to yield any positive samples; 187 samples of commercially produced crab meat did not yield any positive samples. Of the 150 crab plant drains examined, none were positive; of the 316 estuarine water samples taken, only one was positive. Since this reporting period, however, another positive water sample was taken in early April from St. Bernard Parish. In addition to the above sampling, ice houses were examined. Of 20 ice samples, all were negative. Results of the sampling program as of March 8 are shown in Table 2.

### Coordinating Efforts

Even though evidence showed that the problem was with mishandling practices of recreationally caught, home prepared and consumed crabs, Louisiana commercial crab and seafood processors and dealers indicated their sales were being affected. Retailers and restaurants were especially affected. As the problem developed, obvious confusion and gross misunderstanding existed. For example, daily accounts of the situation in many Louisiana newspapers and television and radio broadcasts contributed toward an emotionally involved public. The fact that the disease was not associated with commercially processed product did not surface in the numerous media reportings. Consumers were not aware of exactly how the disease was transmitted or how it could be controlled, except that some people who had eaten crabs had become ill from cholera. Consumers often associated cholera with diseases such as the plague. In addition, Federal and State health officials did not agree on methods of controlling the problem. The crabbing industry was not informed of developments, and there was no mechanism to do so.

In order to better coordinate the efforts of all parties involved, the Louisiana Cooperative Extension Service, in cooperation with the Louisiana Sea Grant Program hosted a meeting. The purpose of the meeting was to coordinate the scientific efforts of the seafood industry, academia, agencies of the State of Louisiana and Federal agencies to define and resolve the cholera problem in Louisiana. The first meeting was called shortly after the reported FDA seizure in Baltimore in October. These meetings have been held on a monthly basis since that time. As a result of these meetings, research proposals have been identified and initiated. Very generally, these proposals involve environmental sampling and

TABLE 2

## SUMMARY OF SPECIMENS EXAMINED — CHOLERA INVESTIGATION — LOUISIANA 1978

As of 3/8/79

Parishes:	Live Crabs		Fresh Shrimp		Raw Oysters		Crab Meat		Sewage Swabs		Septic Tanks		Crab Plant Drains		Estuarine Water		Ice		People*	
	Total	Pos.	Total	Pos.	Total	Pos.	Total	Pos.	Total	Pos.	Total	Pos.	Total	Pos.	Total	Pos.	Total	Pos.	Total	Pos.
Acadia							12		22		7									
Ascension									12											
Assumption	48						56		11											
Calcasieu	4		4		4				87		12		5		4		3		114	
Cameron	49		23		10		2		16		4				41		2			
E. Baton Rouge									20		6									
Iberia					7				34						3		4		75	
Iberville									12											
Jefferson	62		8		4		25		87				37						201	
Jefferson Davis									64		3									
Lafayette	1								87		1								556	11
Lafourche	24						25		16				14				3		318	
Livingston																				
Orleans	57		3		7		7		33				11						702	
Plaquemines	2				4															
St. Bernard	42		3		16		11		15				18							
St. Charles	12						3		5				5							
St. John	3								7											
St. Martin	22						17		22		8		15				1			
St. Mary	43		2		2		15		200		6		27				7		23	
St. Tammany	5				1				23		5								181	
Tangipahoa	8				4		2						3							
Terrebonne	18		1		5		11		20				15						88	
Vermilion	91		65	1	11		1		341		11				268	1			210	
W. Baton Rouge									4											
Total	491		109	1	75		187		1138		76	4**	150		316	1	20		2468	11

\* Recorded by location of laboratory processing specimens, not by parish residence of patient.

\*\*All 4 septic tank swabs which were positive are those directly attributable to patients.

NB: Estimated number tested: crabs—2455; shrimp—1448; oysters—923 (These numbers are probably somewhat lower than what was actually tested since some samples were not submitted with the total number of specimens mentioned in each sample and were treated as being one or two in number.)

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Epidemiology Unit

